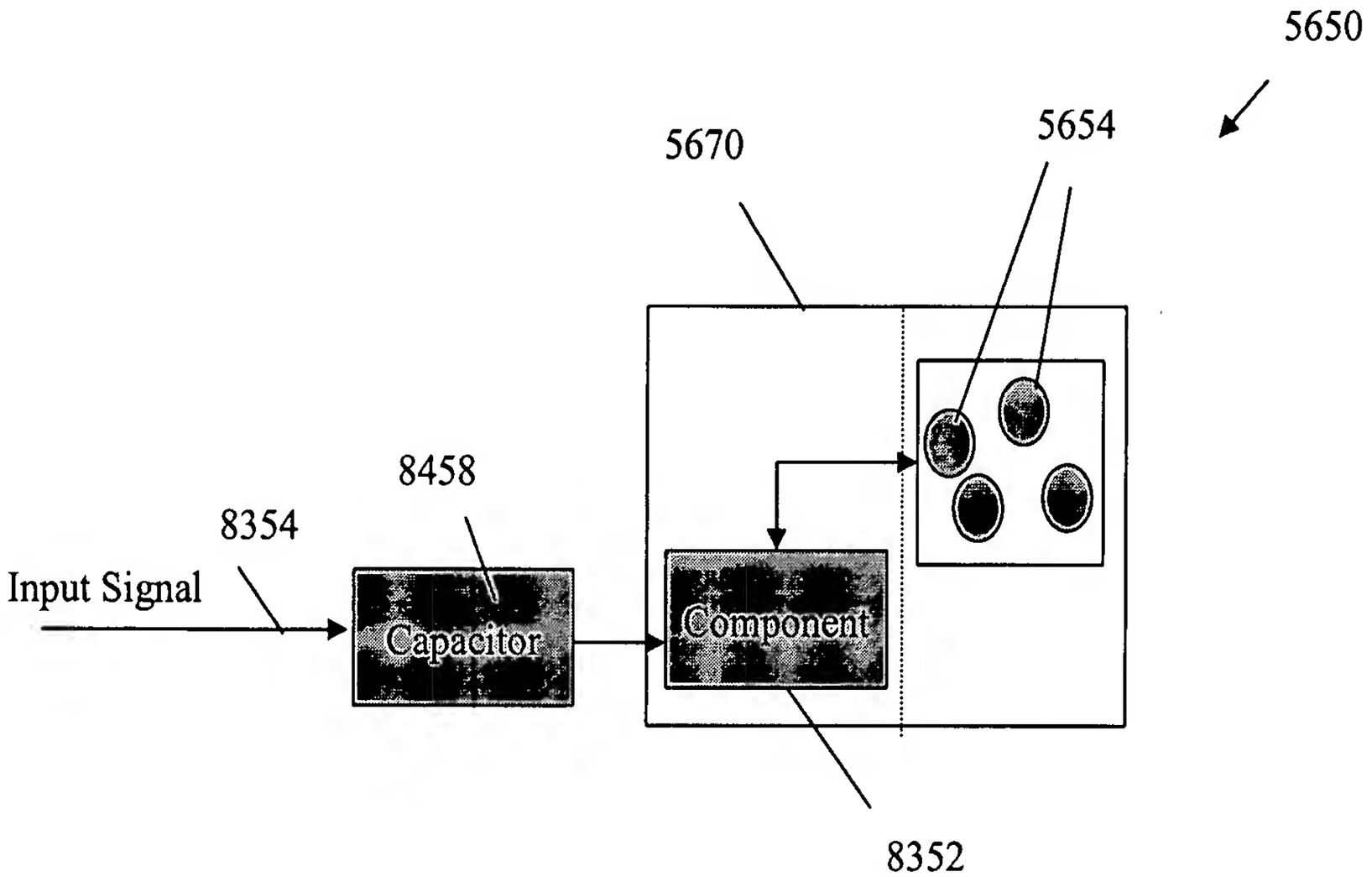
5650
5670
5654
8458
8354
Input Signal
Capacitor
Component
8352

Fig. 84

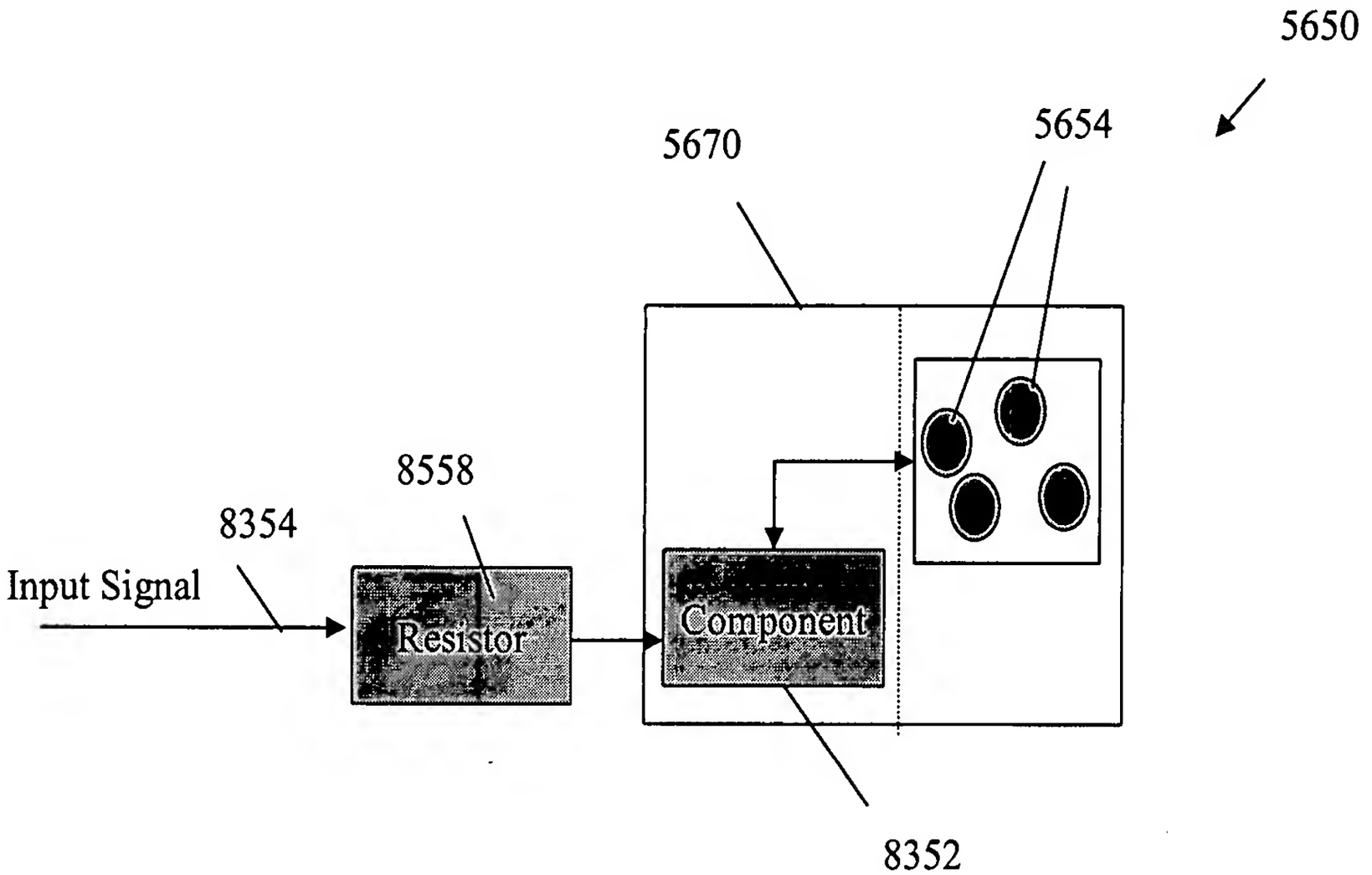
5650
5670
5654
8558
8354
Input Signal
Resistor
Component
8352

Fig. 85

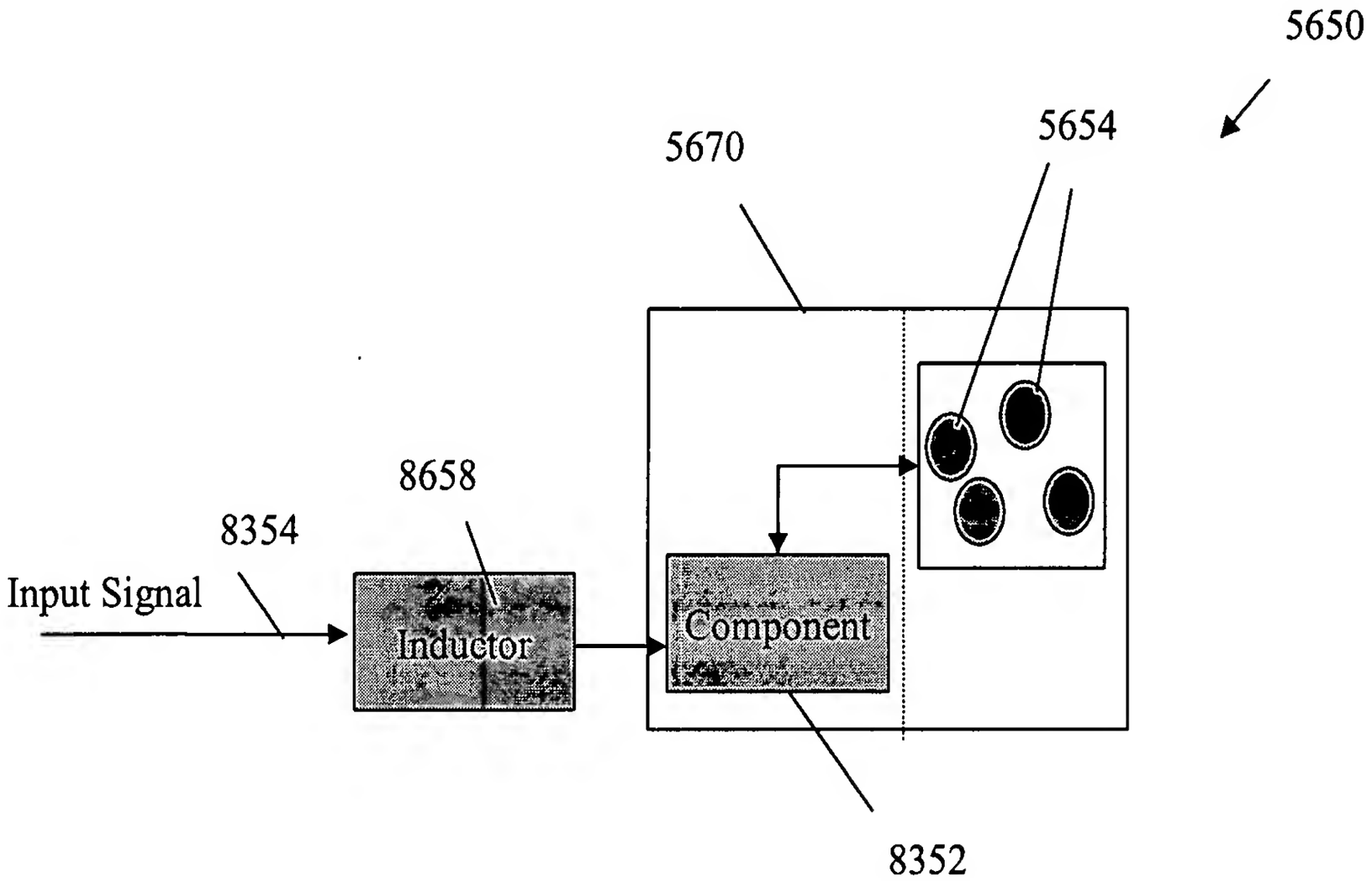
5650
5670
5654
8658
8354
Input Signal
Inductor
Component
8352

Fig. 86

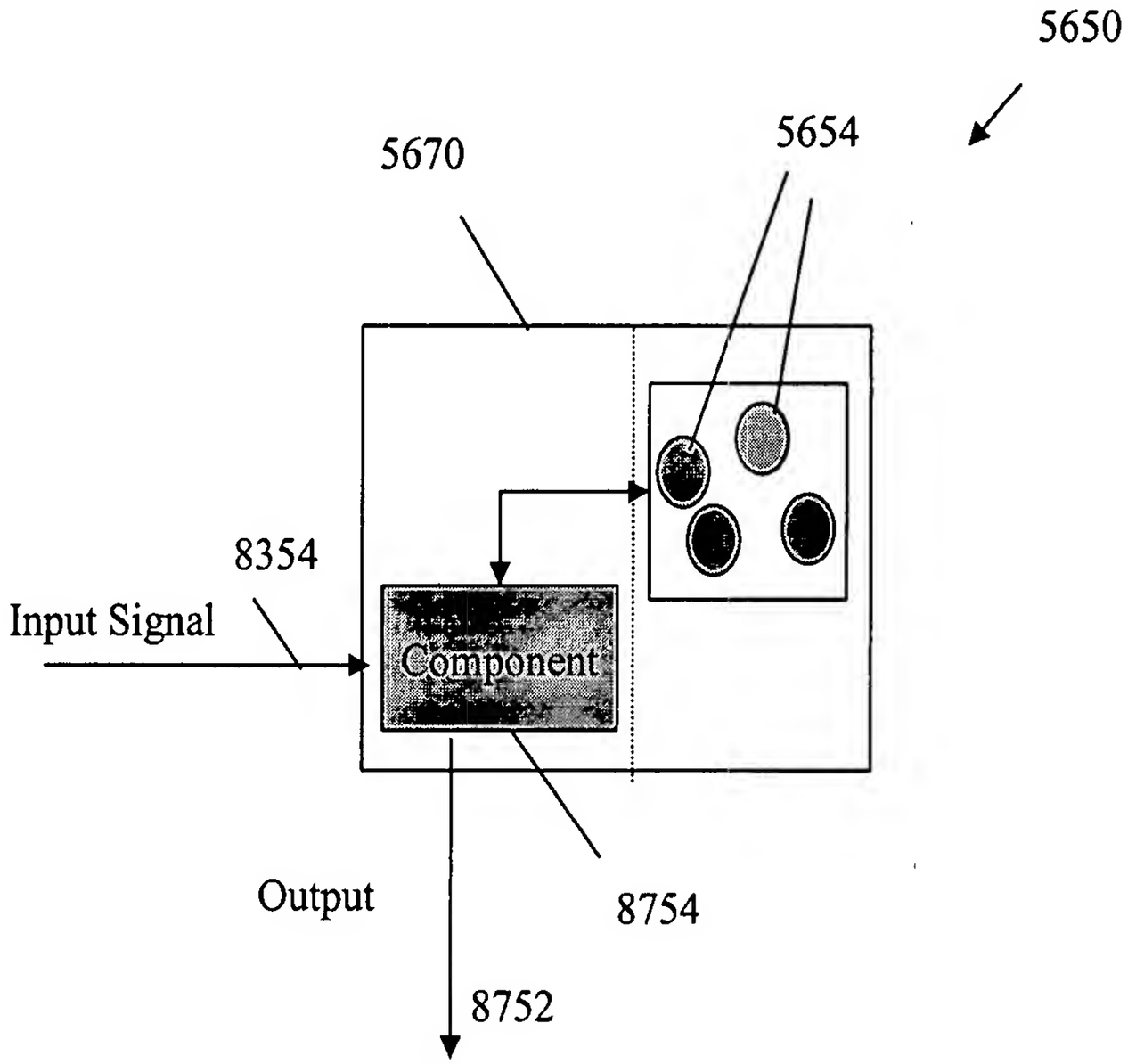
5650
5670
5654
8354
Input Signal
Component
Output
8754
8752

Fig. 87

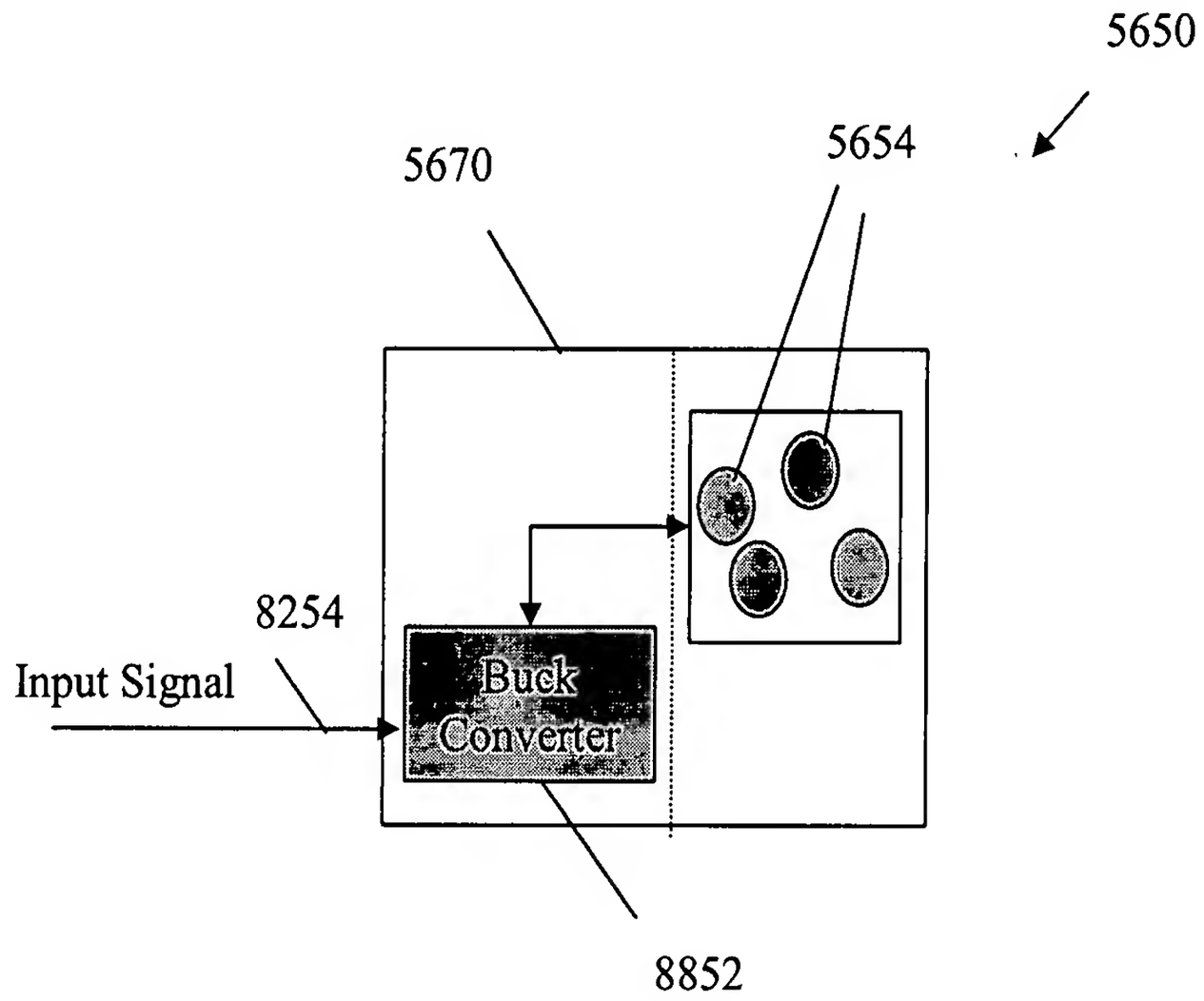

Fig. 88

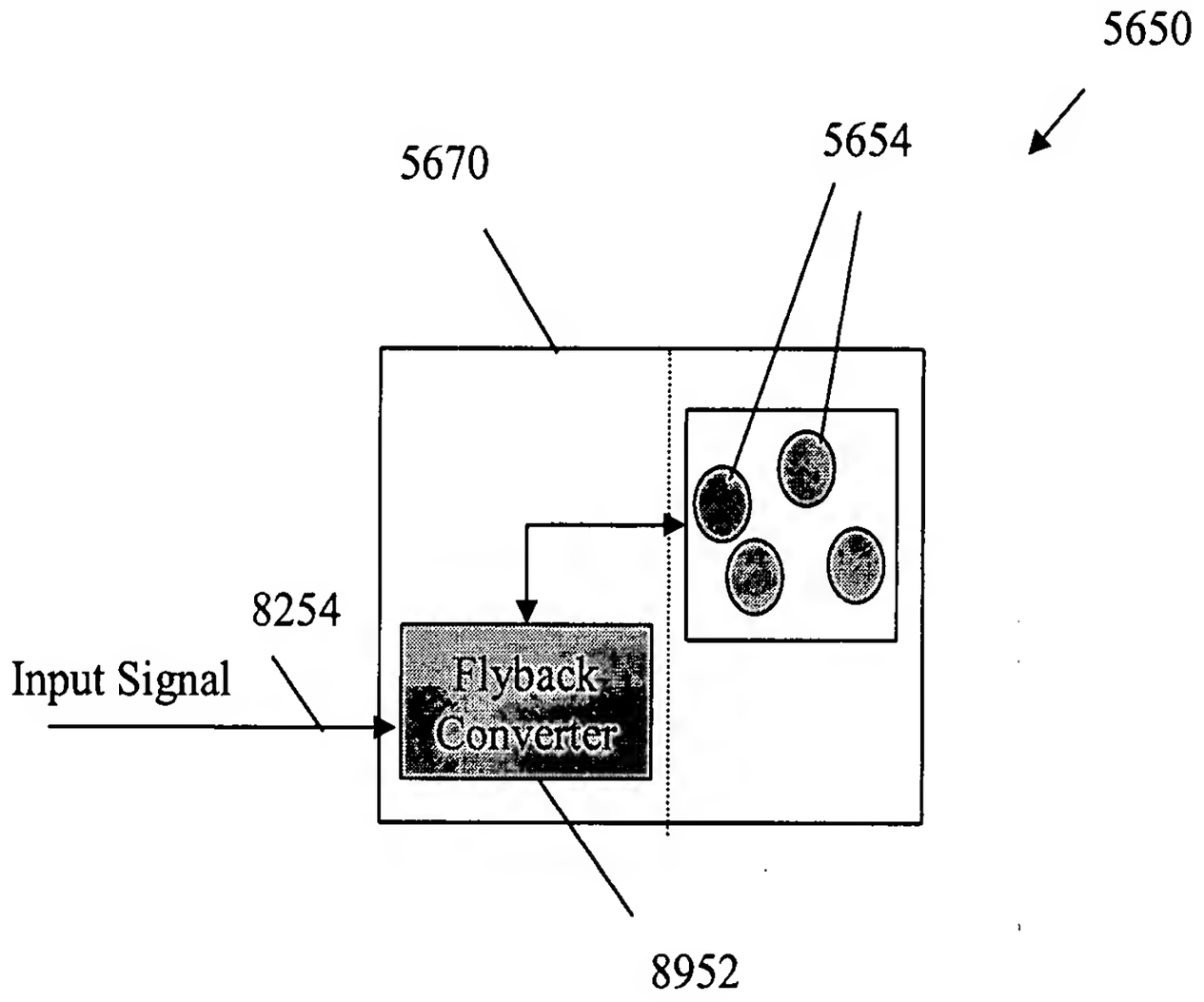

Fig. 89

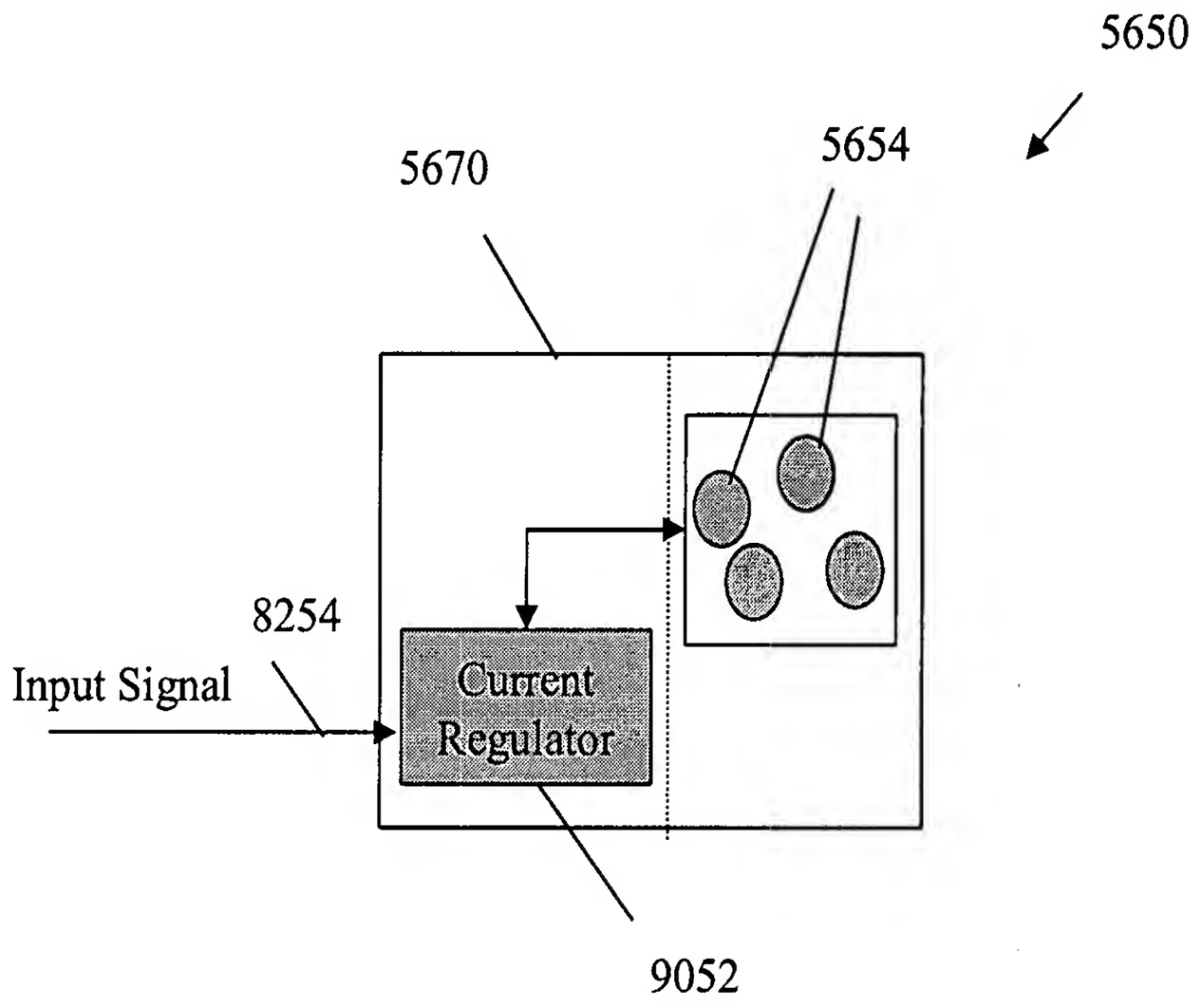

Fig. 90

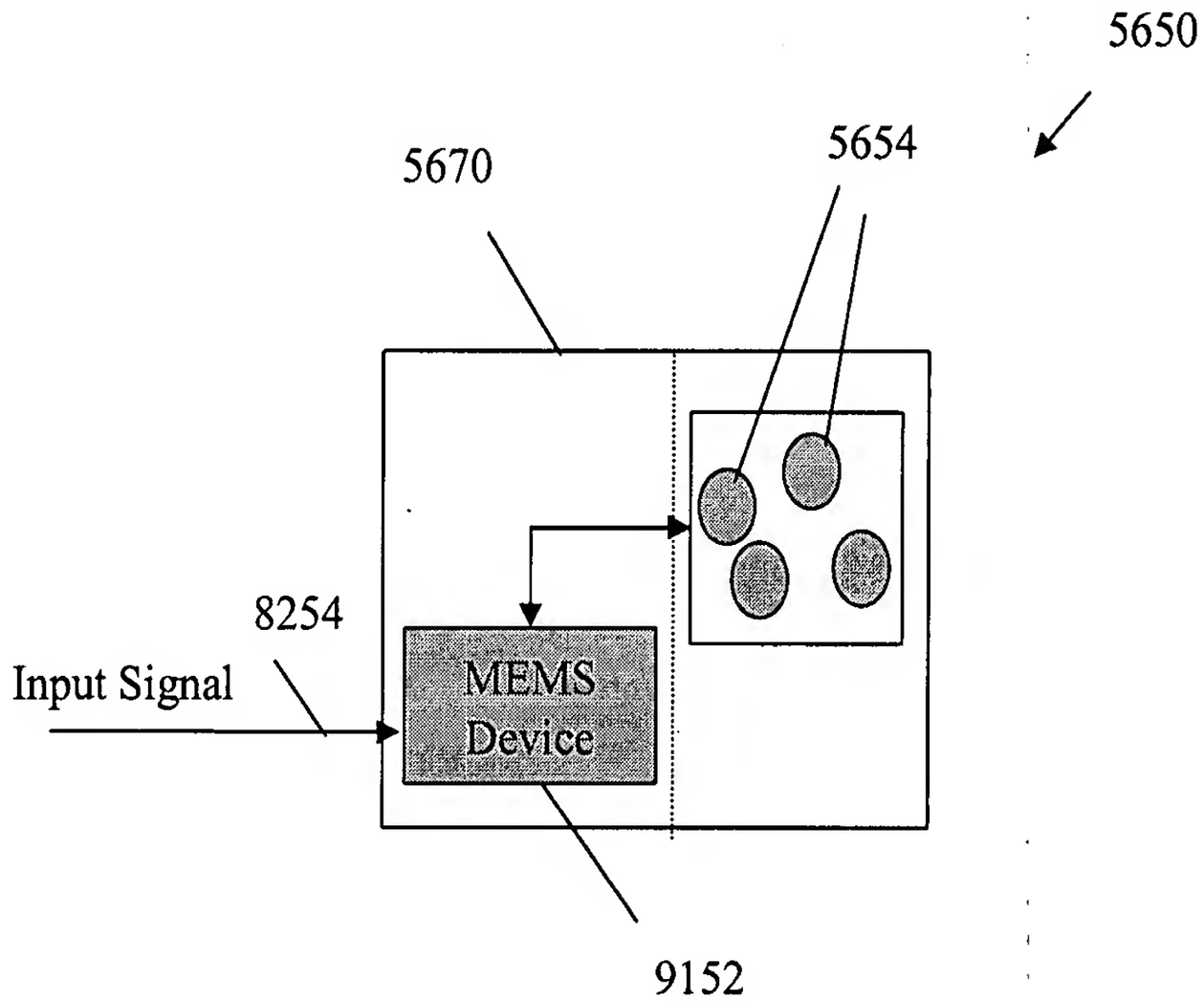

Fig. 91

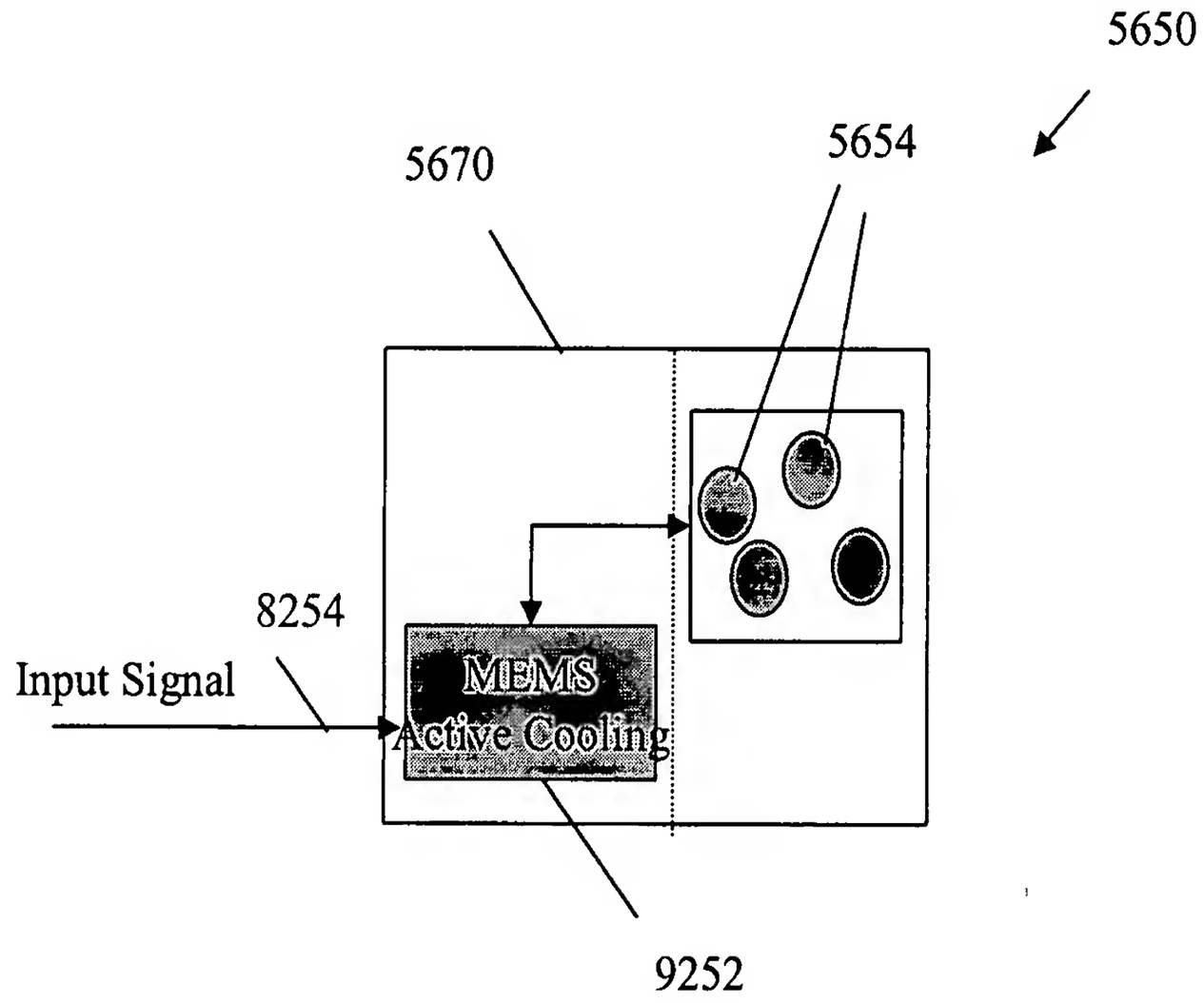

Fig. 92

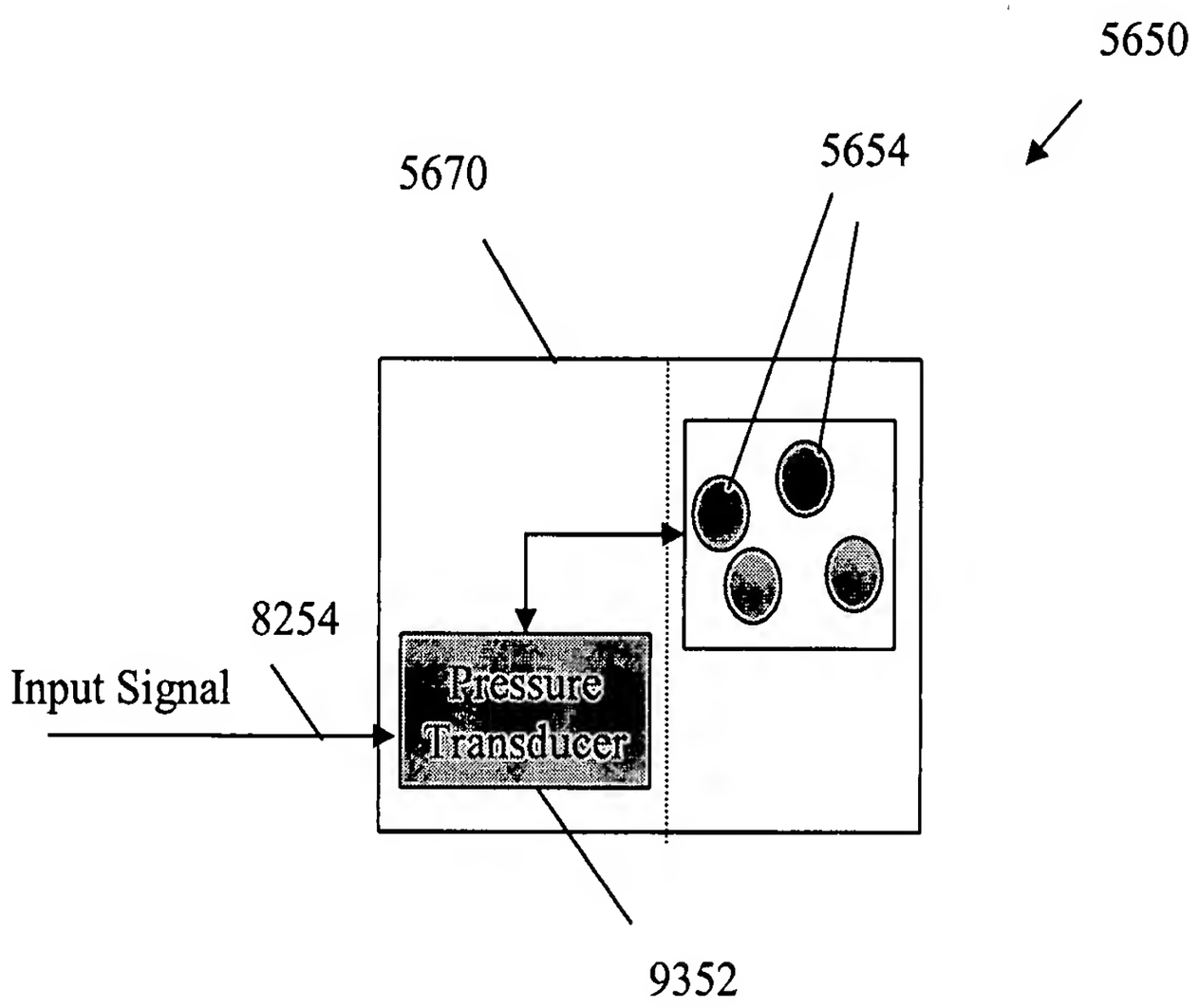

Fig. 93

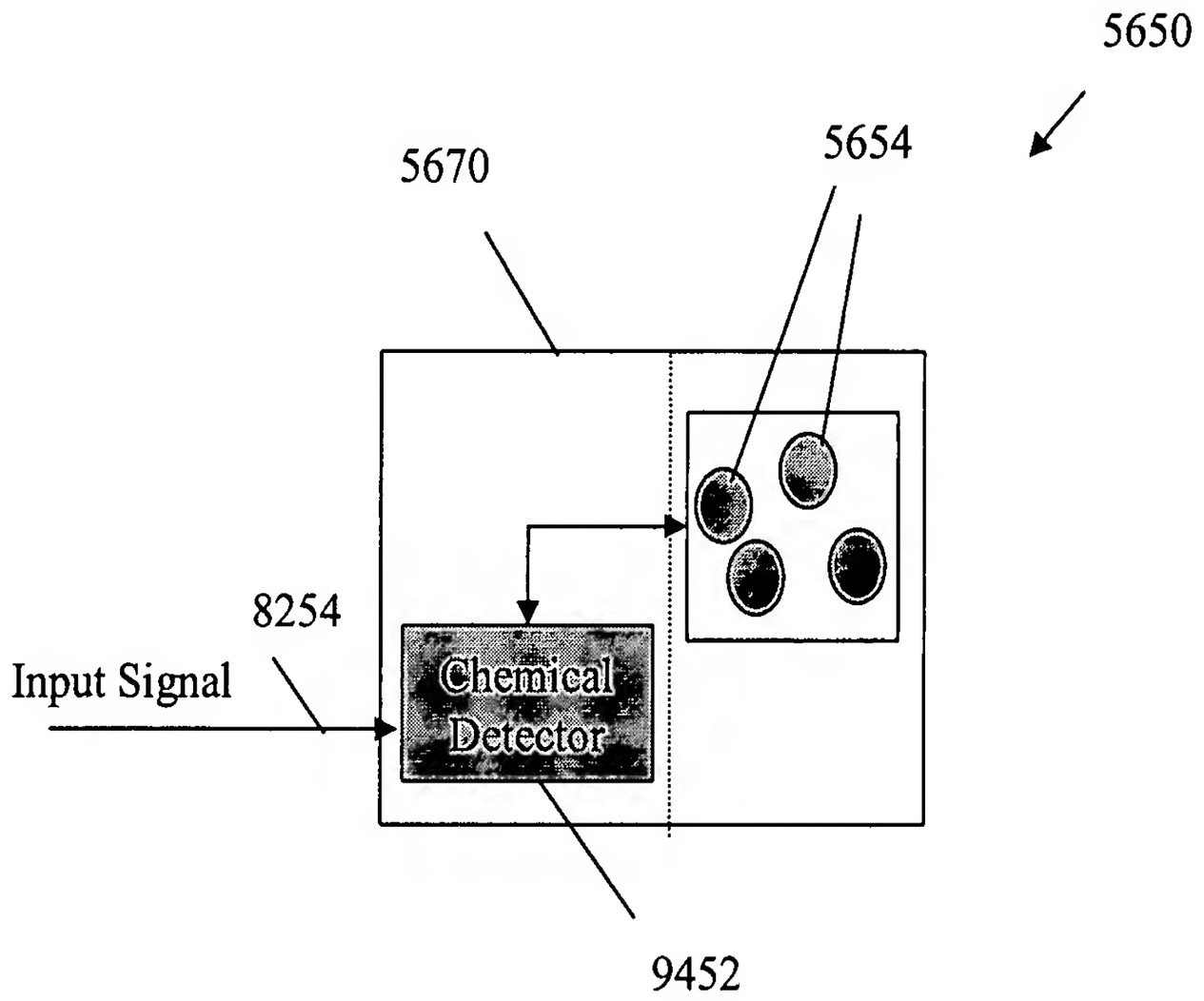

Fig. 94

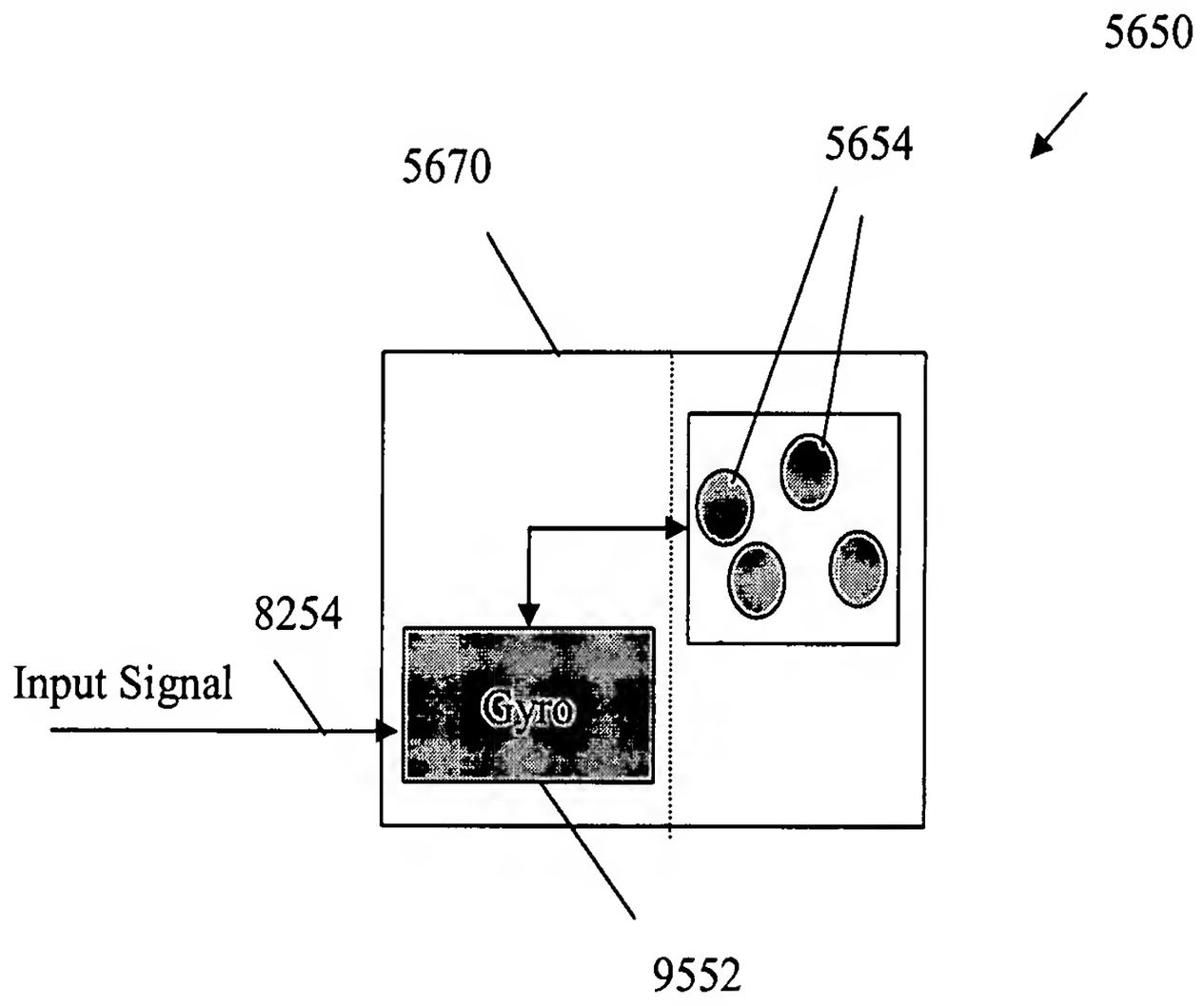

Fig. 95

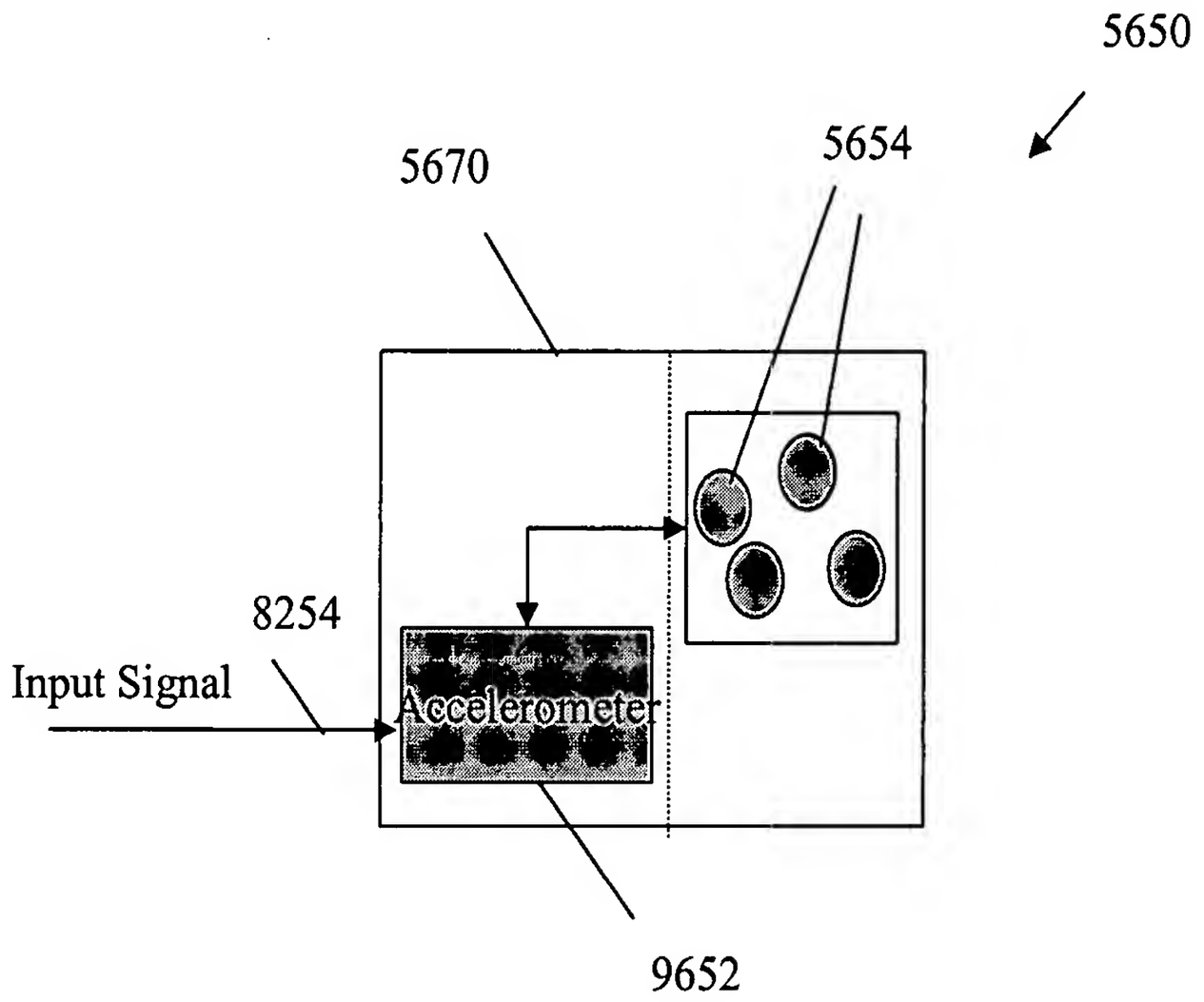

Fig. 96

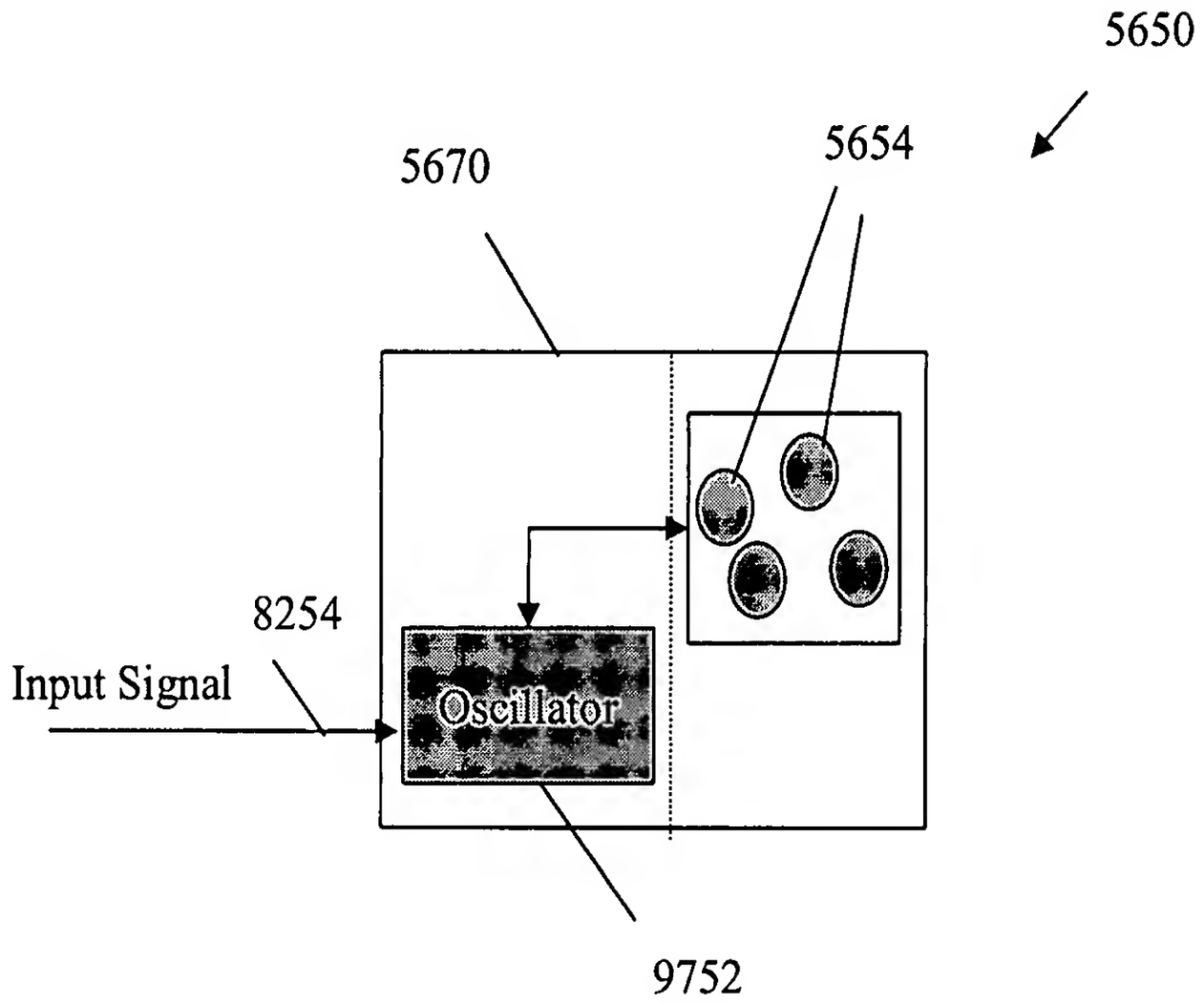

Fig. 97

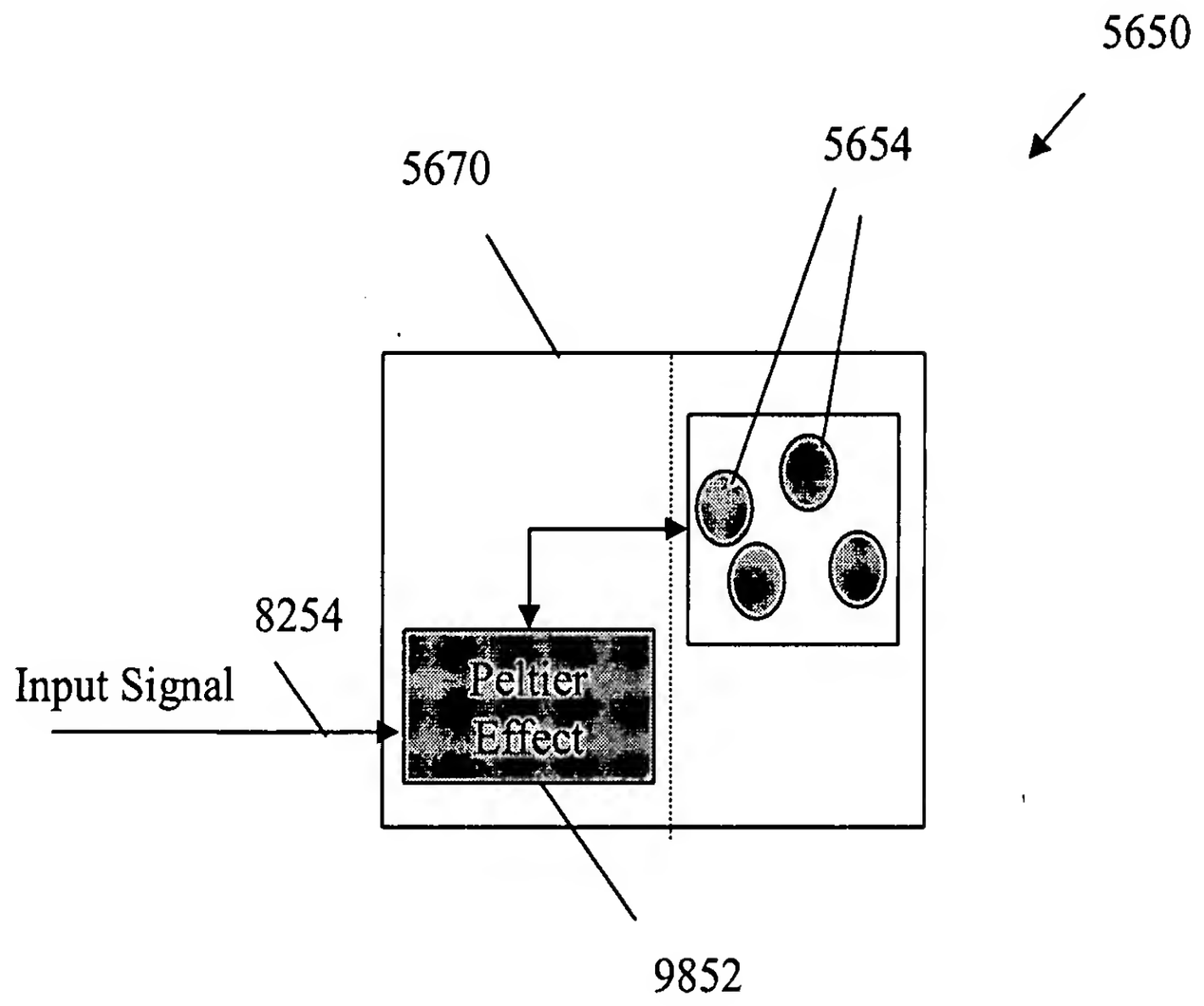

Fig. 98

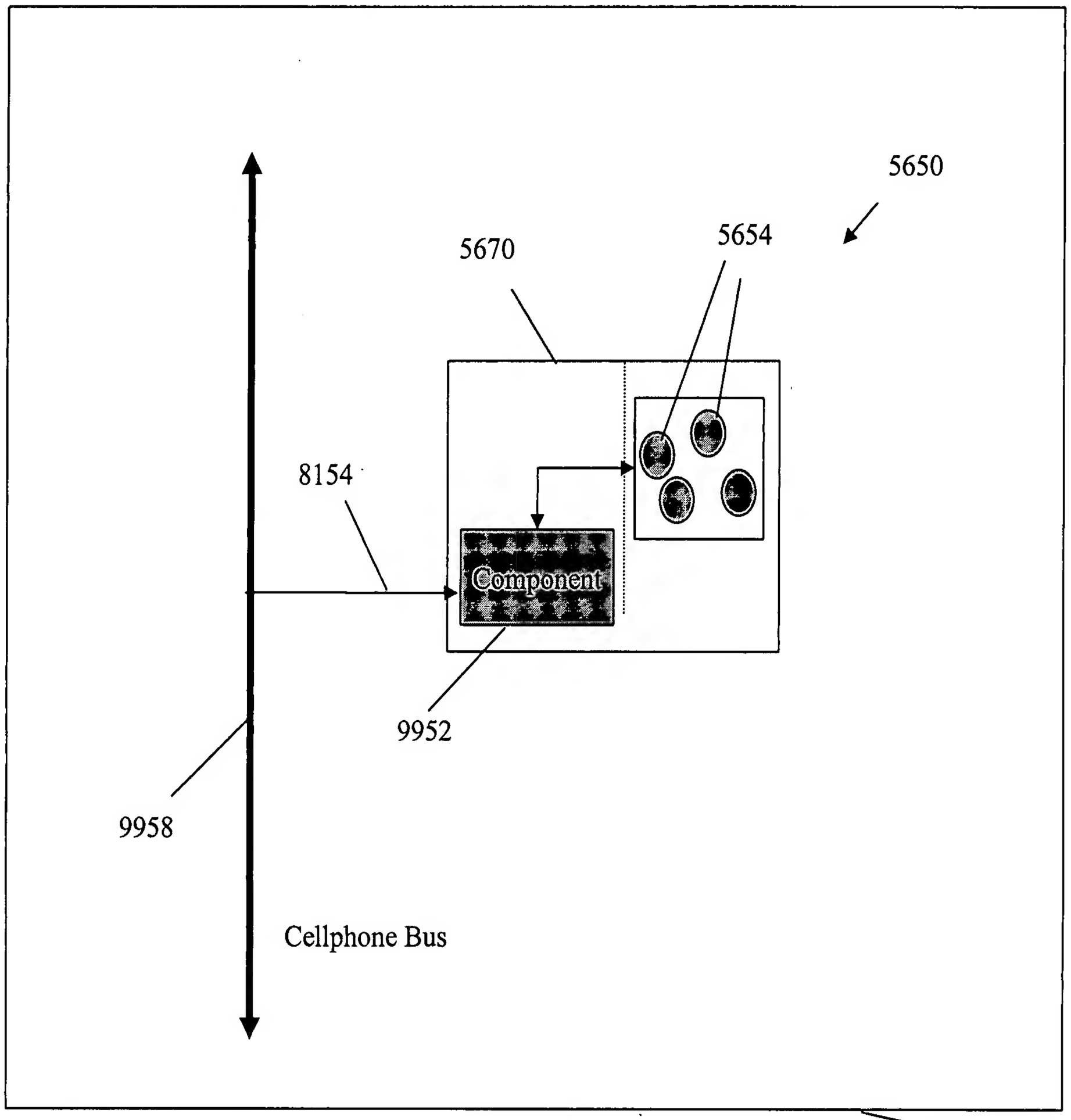

Fig. 99

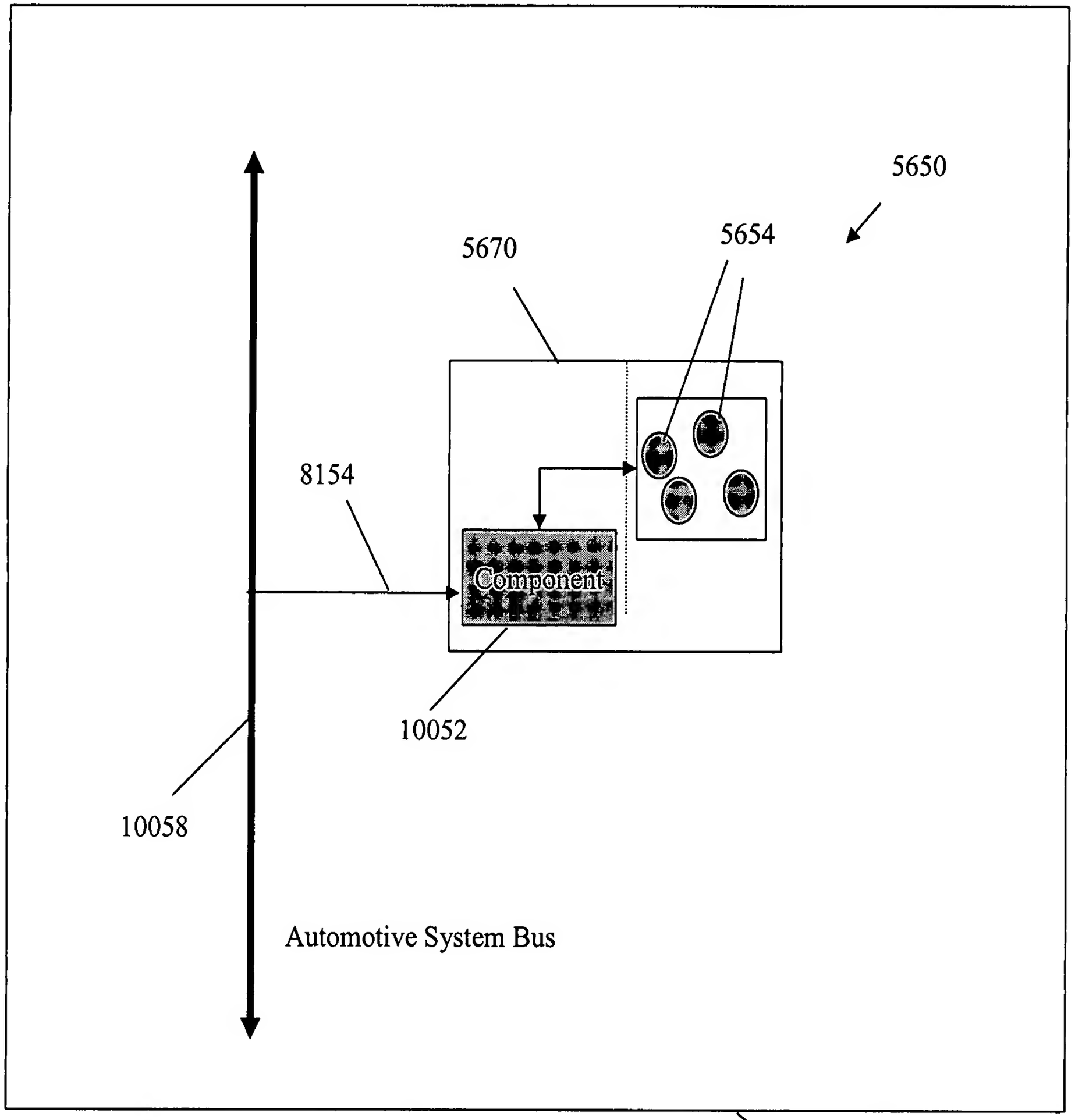
5650
5670
5654
8154
Component
10052
10058
Automotive System Bus
10060

Fig. 100

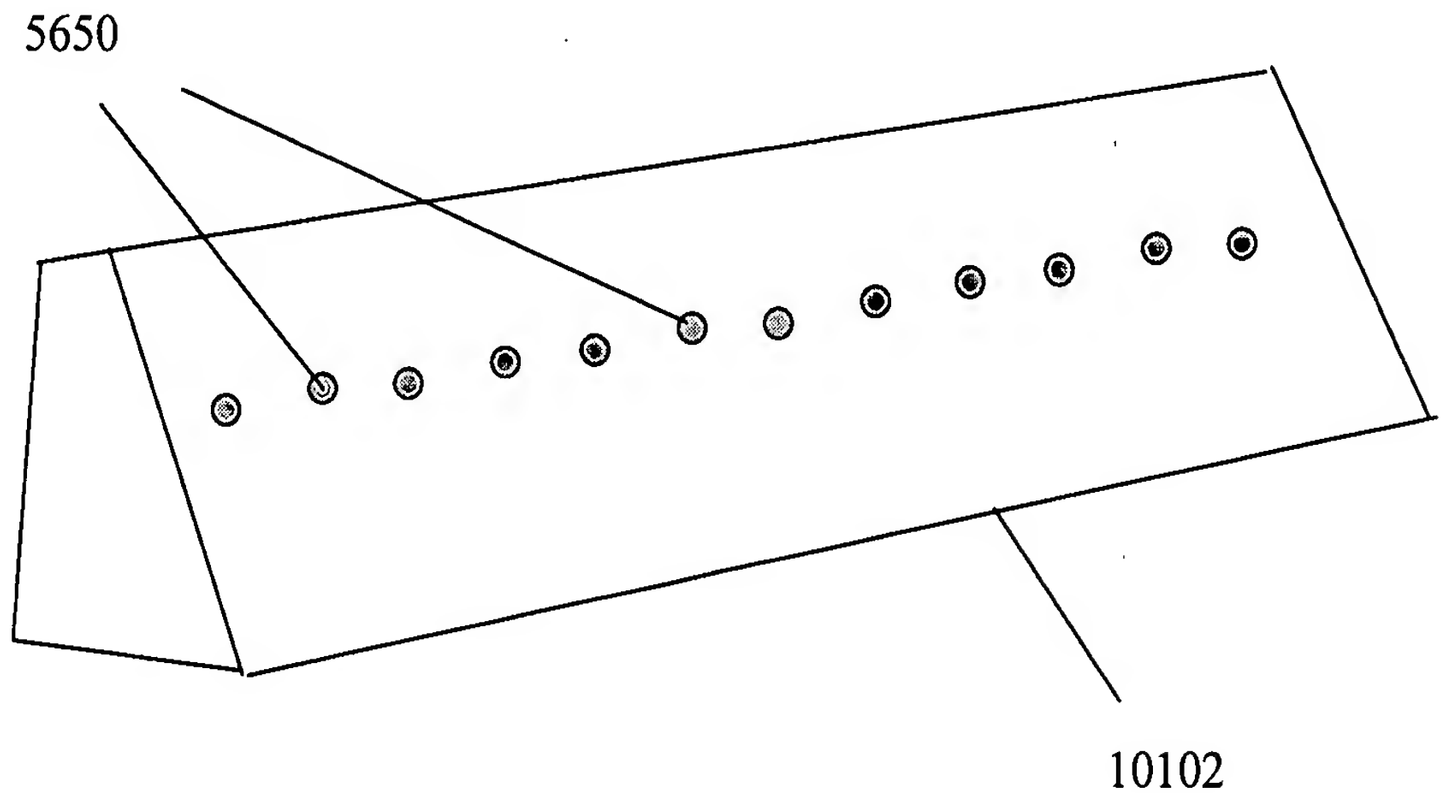

Fig. 101

PATENT APPLICATION SERIAL NO. _____

U.S. DEPARTMENT OF COMMERCE
PATENT AND TRADEMARK OFFICE
FEE RECORD SHEET

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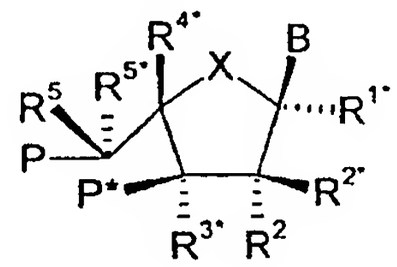
PTO-1556
(5/87)

*U.S. Government Printing Office: 2002 — 489-267/69033

so far, it is preferred that the biradical constituting R^{2*} and R^{4*} forms a two atom bridge, *i.e.* the biradical forms a five membered ring with the furanose ring (X=O).

In one embodiment of the present invention the biradical is $-(CH_2)_{2-4}-$.

For these interesting embodiments, it is preferred that the L-ribo-LNA(s) has/have the following general formula Ia.



Ia

Also interesting as a separate aspect of the present invention is the variant of formula Ia where B is in the "β-configuration".

The oligomers according to the invention typically comprise 1-10000 L-ribo-LNA(s) of the general formula I (or of the more detailed general formula Ia) and 0-10000 nucleosides selected from naturally occurring nucleosides and nucleoside analogues. The sum of the number of nucleosides and the number of L-ribo-LNA(s) (n) is at least 2, preferably at least 3, in particular at least 5, especially at least 7, such as in the range of 2-15000, preferably in the range of 2-100, such as 3-100, in particular in the range of 2-50, such as 3-50 or 5-50 or 7-50.

It has been found that partly L-ribo-LNA modified oligomers hybridise strongly (with increasing affinity) to DNA and RNA. It is presently believed that fully L-ribo-LNA modified oligomers and oligomers consisting of L-ribo-LNA monomers together with other L-ribo-configurated nucleotide analogues, will give rise to comparable hybridisation properties.

In the present context, the term "nucleoside" means a glycoside of a heterocyclic base. The term "nucleoside" is used broadly as to include non-naturally occurring nucleosides, naturally occurring nucleosides as well as other nucleoside analogues.

Illustrative examples of nucleosides are ribonucleosides comprising a ribose moiety as well as deoxyribonuclesides comprising a deoxyribose moiety. With respect to the bases of such nucleosides, it should be understood that this may be any of the naturally

occurring bases, *e.g.* adenine, guanine, cytosine, thymine, and uracil, as well as any modified variants thereof or any possible unnatural bases.

When considering the definitions and the known nucleosides (naturally occurring and non-naturally occurring) and nucleoside analogues (including known bi- and tricyclic analogues), it is clear that an oligomer may comprise one or more L-ribo-LNA(s) (which may be identical or different both with respect to the selection of substituent and with respect to selection of biradical) and one or more nucleosides and/or nucleoside analogues. In the present context "oligonucleotide" means a successive chain of nucleosides connected via internucleoside linkages, however, it should be understood that a nucleobase in one or more nucleotide units (monomers) in an oligomer (oligonucleotide) may have been modified with a substituent B as defined above.

The oligomers may be linear, branched or cyclic. In the case of a branched oligomer, the branching points may be located in a nucleoside, in an internucleoside linkage or, in an intriguing embodiment, in an L-ribo-LNA. It is believed that in the latter case, the substituents R^2, and R^{3*} may designate a group P* designating an internucleoside linkage to a preceding monomer, in particular, R^2 designate a further P^*.

As mentioned above, the L-ribo-LNA(s) of an oligomer are connected with other monomers via an internucleoside linkage. In the present context, the term "internucleoside linkage" means a linkage consisting of 2 to 4, preferably 3, groups/atoms selected from $-CH_2-$, -O-, -S-, $-NR^H-$, >C=O, $>C=NR^H$, >C=S, $-Si(R'')_2-$, -SO-, $-S(O)_2-$, $-P(O)_2-$, $-PO(BH_3)-$, -P(O,S)-, $-P(S)_2-$, -PO(R'')-, $-PO(OCH_3)-$, and $-PO(NHR^H)-$, where R^H is selected form hydrogen and C_{1-4}-alkyl, and R'' is selected from C_{1-6}-alkyl and phenyl. Illustrative examples of such internucleoside linkages are $-CH_2-CH_2-CH_2-$, $-CH_2-CO-CH_2-$, $-CH_2-CHOH-CH_2-$, $-O-CH_2-O-$, $-O-CH_2-CH_2-$, $-O-CH_2-CH=$ (including R^5 when used as a linkage to a succeeding monomer), $-CH_2-CH_2-O-$, $-NR^H-CH_2-CH_2-$, $-CH_2-CH_2-NR^H-$, $-CH_2-NR^H-CH_2-$, $-O-CH_2-CH_2-NR^H-$, $-NR^H-CO-O-$, $-NR^H-CO-NR^H-$, $-NR^H-CS-NR^H-$, $-NR^H-C(=NR^H)-NR^H-$, $-NR^H-CO-CH_2-NR^H-$, -O-CO-O-, $-O-CO-CH_2-O-$, $-O-CH_2-CO-O-$, $-CH_2-CO-NR^H-$, $-O-CO-NR^H-$, $-NR^H-CO-CH_2-$, $-O-CH_2-CO-NR^H-$, $-O-CH_2-CH_2-NR^H-$, -CH=N-O-, $-CH_2-NR^H-O-$, $-CH_2-O-N=$ (including R^5 when used as a linkage to a succeeding monomer), $-CH_2-O-NR^H-$, $-CO-NR^H-CH_2-$, $-CH_2-NR^H-O-$, $-CH_2-NR^H-CO-$, $-O-NR^H-CH_2-$, $-O-NR^H-$, $-O-CH_2-S-$, $-S-CH_2-O-$, $-CH_2-CH_2-S-$, $-O-CH_2-CH_2-S-$, $-S-CH_2-CH=$ (including R^5 when used as a linkage to a succeeding monomer), $-S-CH_2-CH_2-$, $-S-CH_2-$

CH_2-O-, -S-CH_2-CH_2-S-, -CH_2-S-CH_2-, -CH_2-SO-CH_2-, -CH_2-SO_2-CH_2-, -O-SO-O-, -O-$S(O)_2$-O-, -O-$S(O)_2$-CH_2-, -O-$S(O)_2$-NR^H-, -NR^H-$S(O)_2$-CH_2-, -O-$S(O)_2$-CH_2-, -O-$P(O)_2$-O-, -O-P(O,S)-O-, -O-$P(S)_2$-O-, -S-$P(O)_2$-O-, -S-P(O,S)-O-, -S-$P(S)_2$-O-, -O-$P(O)_2$-S-, -O-P(O,S)-S-, -O-$P(S)_2$-S-, -S-$P(O)_2$-S-, -S-P(O,S)-S-, -S-$P(S)_2$-S-, -O-PO(R")-O-, -O-$PO(OCH_3)$-O-, -O-$PO(OCH_2CH_3)$-O-, -O-$PO(OCH_2CH_2S$-R)-O-, -O-$PO(BH_3)$-O-, -O-$PO(NHR^N)$-O-, -O-$P(O)_2$-NR^H-, -NR^H-$P(O)_2$-O-, -O-P(O,NR^H)-O-, -CH_2-$P(O)_2$-O-, -O-$P(O)_2$-CH_2-, and -O-Si(R")$_2$-O-; among which -CH_2-CO-NR^H-, -CH_2-NR^H-O-, -S-CH_2-O-, -O-$P(O)_2$-O-, -O-P(O,S)-O-, -O-$P(S)_2$-O-, -NR^H-$P(O)_2$-O-, -O-P(O,NR^H)-O-, -O-PO(R")-O-, -O-$PO(CH_3)$-O-, and -O-$PO(NHR^N)$-O-, where R^H is selected form hydrogen and C_{1-4}-alkyl, and R" is selected from C_{1-6}-alkyl and phenyl, are especially preferred. Further illustrative examples are given in Mesmaeker et. al., Current Opinion in Structural Biology 1995, 5, 343-355. The left-hand side of the internucleoside linkage is bound to the 5-membered ring as substituent P', whereas the right-hand side is bound to the 5'-position of a preceding monomer.

It is also clear from the above that the group P may also designate a 5'-terminal group in the case where the L-ribo-LNA in question is the 5'-terminal monomer. Examples of such 5'-terminal groups are hydrogen, hydroxy, optionally substituted C_{1-6}-alkyl, optionally substituted C_{1-6}-alkoxy, optionally substituted C_{1-6}-alkylcarbonyloxy, optionally substituted aryloxy, monophosphate, diphosphate, triphosphate, and -W-A', wherein W is selected from -O-, -S-, and -N(R^H)- where R^H is selected from hydrogen and C_{1-6}-alkyl, and where A' is selected from DNA intercalators, photochemically active groups, thermochemically active groups, chelating groups, reporter groups, and ligands (where the latter groups may include a spacer as defined for the substituent B).

In the present description and claims, the terms "monophosphate", "diphosphate", and "triphosphate" mean groups of the formula: -O-$P(O)_2$-O^-, -O-$P(O)_2$-O-$P(O)_2$-O^-, and -O-$P(O)_2$-O-$P(O)_2$-O-$P(O)_2$-O^-, respectively.

In a particularly interesting embodiment, the group P designates a 5'-terminal groups selected from monophosphate, diphosphate and triphosphate. Especially the triphosphate variant of formula II is interesting as a substrate,such as for enzymes especially for those active on nucleic acids.

Analogously, the group P* may designate a 3'-terminal group in the case where the L-ribo-LNA in question is the 3'-terminal monomer. Examples of such 3'-terminal groups are hydrogen, hydroxy, optionally substituted C_{1-6}-alkoxy, optionally substituted C_{1-6}-alkylcarbonyloxy, optionally substituted aryloxy, and -W-A', wherein W is selected from -O-, -S-, and -N(R^H)- where R^H is selected from hydrogen and C_{1-6}-alkyl, and where A' is selected from DNA intercalators, photochemically active groups, thermochemically active groups, chelating groups, reporter groups, and ligands (where the latter groups may include a spacer as defined for the substituent B).

In a preferred embodiment of the present invention, the oligomer has the following formula III:

$$G\text{-}[Nu\text{-}L]_{n(0)}\text{-}\{[(L\text{-ribo-LNA})\text{-}L]_{m(q)}\text{-}[Nu\text{-}L]_{n(q)}\}_q\text{-}G^* \quad III$$

wherein

q is 1-50;

each of n(0), .., n(q) is independently 0-10000;

each of m(1), .., m(q) is independently 1-10000;

with the proviso that the sum of n(0), .., n(q) and m(1), .., m(q) is 2-15000;

G designates a 5'-terminal group;

each Nu independently designates a nucleoside selected from naturally occurring nucleosides and nucleoside analogues;

each L-ribo-LNA independently designates a nucleoside analogue;

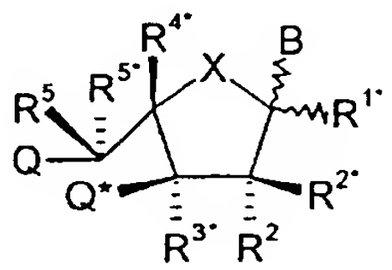
each L independently designates an internucleoside linkage between two groups selected from Nu and L-ribo-LNA, or L together with G* designates a 3'-terminal group; and

each (L-ribo-LNA)-L independently designates a nucleoside analogue of the general formula I as defined above, or preferably of the general formula Ia as defined above.

Within this embodiment, as well as generally, the present invention provides the intriguing possibility of including L-ribo-LNAs with different nucleobases, in particular both nucleobases selected from thymine, cytosine and uracil and nucleobases selected from adenine and guanine. The oligomer may comprise, in one embodiment, at least one L-ribo-LNA wherein B (in fromula I or Ia) is selected from the group comprising adenine and guanine and at least one L-ribo-LNA wherein B is selected from the group comprising thymine, cytosine and uracil.

Apart from the oligomers defined above, the present invention also provides monomeric L-ribo-LNAs useful, *e.g.*, in the preparation of oligomers, as substrates for, *e.g.*, nucleic acid polymerases, polynucleotide kinases, terminal transferases, and as therapeutical agents, see further below. The monomeric L-ribo-LNAs correspond in the overall structure (especially with respect to the possible biradicals) to the L-ribo-LNAs defined as constituents in oligomers, however with respect to the groups P and P*, the monomeric L-ribo-LNAs differ slightly as will be explained below. Furthermore, the monomeric L-ribo-LNAs may comprise functional group protecting groups, especially in the cases where the monomeric L-ribo-LNAs are to be incorporated into oligomers by chemical synthesis.

The invention furthermore relates to monomeric L-ribo-LNA nucleosides (L-ribo-LNAs) of the general formula II:



II

wherein the substituent B is selected from nucleobases, DNA intercalators, photochemically active groups, thermochemically active groups, chelating groups, reporter groups, and ligands; X is selected from -O-, -S-, -N(R^{N*})-, and -C(R^6R^{6*})-, preferably from -O-, -S-, and -N(R^{N*})-;

each of Q and Q* is independently selected from hydrogen, azido, halogen, cyano, nitro, hydroxy, Prot-O-, Act-O-, mercapto, Prot-S-, Act-S-, C_{1-6}-alkylthio, amino, Prot-N(R^H)-, Act-N(R^H)-, mono- or di(C_{1-6}-alkyl)amino, optionally substituted C_{1-6}-alkoxy, optionally substituted C_{1-6}-alkyl, optionally substituted C_{2-6}-alkenyl, optionally substituted C_{2-6}-alkenyloxy, optionally substituted C_{2-6}-alkynyl, optionally substituted C_{2-6}-alkynyloxy, monophosphate, diphosphate, triphosphate, DNA intercalators, photochemically active groups, thermochemically active groups, chelating groups, reporter groups, ligands, carboxy, sulphono, hydroxymethyl, Prot-O-CH_2-, Act-O-CH_2-, aminomethyl, Prot-N(R^H)-CH_2-, Act-N(R^H)-CH_2-, carboxymethyl, sulphonomethyl, where Prot is a protection group for -OH, -SH, and -NH(R^H), respectively, Act is an activation group for -OH, -SH, and -NH(R^H), respectively, and R^H is selected from hydrogen and C_{1-6}-alkyl;

R^{2*} and R^{4*} together designate a biradical selected from -O-, -S-, -N(R^*)-, -(CR^*R^*)$_{r+s+1}$-, -(CR^*R^*)$_r$-O-(CR^*R^*)$_s$-, -(CR^*R^*)$_r$-S-(CR^*R^*)$_s$-, -(CR^*R^*)$_r$-N(R^*)-(CR^*R^*)$_s$-, -O-(CR^*R^*)$_{r+s}$-O-, -S-(CR^*R^*)$_{r+s}$-O-, -O-(CR^*R^*)$_{r+s}$-S-, -N(R^*)-(CR^*R^*)$_{r+s}$-O-, -O-(CR^*R^*)$_{r+s}$-N(R^*)-, -S-(CR^*R^*)$_{r+s}$-S-, -N(R^*)-(CR^*R^*)$_{r+s}$-N(R^*)-, -N(R^*)-(CR^*R^*)$_{r+s}$-S-, and -S-(CR^*R^*)$_{r+s}$-N(R^*)-; wherein R^* is as defined above for the oligomers; and each of the substituents R^{1*}, R^2, R^{3*}, R^5, and R^{5*}, which are not involved in Q, or Q^*, are as defined above for the oligomers.

The monomeric L-ribo-LNAs also comprise basic salts and acid addition salts thereof.

Furthermore, it should be understood that any chemical group (including any nucleobase), which is reactive under the conditions prevailing in chemical oligonucleotide synthesis, is optionally functional group protected as known in the art. This means that groups such as hydroxy, amino, carboxy, sulphono, and mercapto groups, as well as nucleobases, of a monomeric L-ribo-LNA are optionally functional group protected. Protection (and deprotection) is performed by methods known to the person skilled in the art (see, *e.g.*, Greene, T. W. and Wuts, P. G. M., "Protective Groups in Organic Synthesis", 2nd ed., John Wiley, N.Y. (1991), and M.J. Gait, Oligonucleotide Synthesis, IRL Press, 1984).

Illustrative examples of hydroxy protection groups are optionally substituted trityl (Tr), such as 4,4'-dimethoxytrityl (DMT), 4-monomethoxytrityl (MMT), and trityl, optionally substituted 9-(9-phenyl)xanthenyl (pixyl), optionally substituted ethoxycarbonyloxy, *p*-phenylazophenyloxycarbonyloxy, tetrahydropyranyl (thp), 9-fluorenylmethoxycarbonyl (Fmoc), methoxytetrahydropyranyl (mthp), silyloxy such as trimethylsilyl (TMS), triisopropylsilyl (TIPS), *tert*-butyldimethylsilyl (TBDMS), triethylsilyl (TES), and phenyl-dimethylsilyl, benzyloxycarbonyl or substituted benzyloxycarbonyl ethers such as 2-bromo benzyloxycarbonyl, *tert*-butylethers, alkyl ethers such as methyl ether, acetals (including two hydroxy groups), acyloxy such as acetyl or halogen substituted acetyls, *e.g.* chloroacetyl or fluoroacetyl, isobutyryl, pivaloyl, benzoyl and substituted benzoyl, methoxymethyl (MOM), benzyl ethers or substituted benzyl ethers such as 2,6-dichlorobenzyl (2,6-Cl_2Bzl). Alternatively, the hydroxy group may be protected by attachment to a solid support optionally through a linker.

Illustrative examples of amino protection groups are Fmoc (fluorenylmethoxycarbonyl), BOC (*tert*-butyloxycarbonyl), trifluoroacetyl, allyloxycarbonyl (alloc, AOC), benzyl-oxycarbonyl (Z, Cbz), substituted benzyloxycarbonyls such as 2-chloro benzyloxycarbonyl

((2-ClZ), monomethoxytrityl (MMT), dimethoxytrityl (DMT), phthaloyl, and 9-(9-phenyl)xanthenyl (pixyl).

Illustrative examples of carboxy protection groups are allyl esters, methyl esters, ethyl esters, 2-cyanoethylesters, trimethylsilylethylesters, benzyl esters (Obzl), 2-adamantyl esters (O-2-Ada), cyclohexyl esters (OcHex), 1,3-oxazolines, oxazoler, 1,3-oxazolidines, amides or hydrazides.

Illustrative examples of mercapto protecting groups are trityl (Tr), acetamidomethyl (acm), trimethylacetamidomethyl (Tacm), 2,4,6-trimethoxybenzyl (Tmob), *tert*-butylsulfenyl (S*t*Bu), 9-fluorenylmethyl (Fm), 3-nitro-2-pyridinesulfenyl (Npys), and 4-methylbenzyl (Meb).

Furthermore, it may be necessary or desirable to protect any nucleobase included in a monomeric L-ribo-LNA, especially when the monomeric L-ribo-LNA is to be incorporated in an oligomer according to the invention. In the present context, the term "protected nucleobases" means that the nucleobase in question is carrying a protection group selected among the groups which are well-known for a man skilled in the art (see *e.g.* Protocols for Oligonucleotides and Analogs, vol 20, (Sudhir Agrawal, ed.), Humana Press, 1993, Totowa, NJ; S. L. Beaucage and R. P. Iyer, *Tetrahedron*, 1993, **49**, 6123; S. L. Beaucage and R. P. Iyer, *Tetrahedron*, 1992, **48**, 2223; and E. Uhlmann and A. Peyman, *Chem. Rev.*, **90**, 543.). Illustrative examples are benzoyl, isobutyryl, tert-butyl, tert-butyloxycarbonyl, 4-chloro-benzyloxycarbonyl, 9-fluorenylmethyl, 9-fluorenylmethyloxy-carbonyl, 4-methoxybenzoyl, 4-methoxytriphenylmethyl, optionally substituted triazolo, *p*-toluenesulphonyl, optionally substituted sulphonyl, isopropyl, optionally substituted amidines, optionally substituted trityl, phenoxyacetyl, optionally substituted acyl, pixyl, tetrahydropyranyl, optionally substituted silyl ethers, and 4-methoxybenzyloxycarbonyl. Chapter 1 in "Protocols for oligonucleotide conjugates", Methods in Molecular Biology, vol 26, (Sudhir Agrawal, ed.), Humana Press, 1993, Totowa, NJ. and S. L. Beaucage and R. P. Iyer, *Tetrahedron*, 1992, **48**, 2223 disclose further suitable examples.

In a preferred embodiment, the group B in a monomeric L-ribo-LNA is preferably selected from nucleobases and protected nucleobases.

In an embodiment of the monomeric L-ribo-LNAs according to the present invention, one of Q and Q*, preferably Q*, designates a group selected from Act-O-, Act-S-, Act-N(R^H)-, Act-O-CH_2-, Act-S-CH_2-, Act-N(R^H)-CH_2-, and the other of Q and Q*, preferably Q, designates a group selected from hydrogen, azido, halogen, cyano, nitro, hydroxy, Prot-O-, mercapto, Prot-S-, C_{1-6}-alkylthio, amino, Prot-N(R^H)-, mono- or di(C_{1-6}-alkyl)amino, optionally substituted C_{1-6}-alkoxy, optionally substituted C_{1-6}-alkyl, optionally substituted C_{2-6}-alkenyl, optionally substituted C_{2-6}-alkenyloxy, optionally substituted C_{2-6}-alkynyl, optionally substituted C_{2-6}-alkynyloxy, monophosphate, diphosphate, triphosphate, DNA intercalators, photochemically active groups, thermochemically active groups, chelating groups, reporter groups, ligands, carboxy, sulphono, hydroxymethyl, Prot-O-CH_2-, aminomethyl, Prot-N(R^H)-CH_2-, carboxymethyl, sulphonomethyl, and R^H is selected from hydrogen and C_{1-6}-alkyl.

In the case described above, the group Prot designates a protecting group for -OH, -SH, and -NH(R^H), respectively. Such protection groups are selected from the same as defined above for hydroxy protection groups, mercapto protection group, and amino protection groups, respectively, however taking into consideration the need for a stable and reversible protection group. However, it is preferred that any protection group for -OH is selected from optionally substituted trityl, such as dimethoxytrityl (DMT), monomethoxytrityl (MMT), and trityl, and 9-(9-phenyl)xanthenyl (pixyl), optionally substituted, tetrahydropyranyl (thp) (further suitable hydroxy protection groups for phosphoramidite oligonucleotide synthesis are described in Agrawal, ed. "Protocols for Oligonucleotide Conjugates"; Methods in Molecular Biology, vol. 26, Humana Press, Totowa, NJ (1994) and Protocols for Oligonucleotides and Analogs, vol 20, (Sudhir Agrawal, ed.), Humana Press, 1993, Totowa, NJ), or protected as acetal; that any protection group for -SH is selected from trityl, such as dimethoxytrityl (DMT), monomethoxytrityl (MMT), and trityl, and 9-(9-phenyl)xanthenyl (pixyl), optionally substituted, tetrahydropyranyl (thp) (further suitable mercapto protection groups for phosphoramidite oligonucleotide synthesis are also described in Agrawal (see above); and that any protecting group for -NH(R^H) is selected from trityl, such as dimethoxytrityl (DMT), monomethoxytrityl (MMT), and trityl, and 9-(9-phenyl)xanthenyl (pixyl), optionally substituted, tetrahydropyranyl (thp) (further suitable amino protection groups for phosphoramidite oligonucleotide synthesis are also described by Agrawal (see above).

In the embodiment above, as well as for any monomeric L-ribo-LNAs defined herein, Act designates an activation group for -OH, -SH, and -NH(R^H), respectively. Such activation groups are, *e.g.*, selected from optionally substituted O-phosphoramidite, optionally substituted O-phosphortriester, optionally substituted O-phosphordiester, optionally substituted H-phosphonate, and optionally substituted O-phosphonate.

In the present context, the term "phosphoramidite" means a group of the formula -P(OR^x)-$N(R^y)_2$, wherein R^x designates an optionally substituted alkyl group, *e.g.* methyl, 2-cyanoethyl, or benzyl, and each of R^y designate optionally substituted alkyl groups, *e.g.* ethyl or isopropyl, or the group -$N(R^y)_2$ forms a morpholino group (-$N(CH_2CH_2)_2O$). R^x preferably designates 2-cyanoethyl and the two R^y are preferably identical and designate isopropyl. Thus, an especially relevant phosphoramidite is *N,N*-diisopropyl-*O*-(2-cyanoethyl)phosphoramidite.

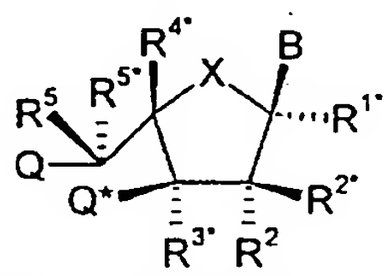
It should be understood that the protecting groups used herein for a single monomeric L-ribo-LNA or several monomeric L-ribo-LNAs may be selected so that when this/these L-ribo-LNA(s) are incorporated in an oligomer according to the invention, it will be possible to perform either a simultaneous deprotection or a sequential deprotection of the functional groups. The latter situation opens for the possibility of regioselectively introducing one or several "active/functional" groups such as DNA intercalators, photochemically active groups, thermochemically active groups, chelating groups, reporter groups, and ligands, where such groups may be attached via a spacer as described above.

In a preferred embodiment, Q is selected from hydrogen, azido, halogen, cyano, nitro, hydroxy, Prot-O-, mercapto, Prot-S-, C_{1-6}-alkylthio, amino, Prot-N(R^H)-, mono- or di(C_{1-6}-alkyl)amino, optionally substituted C_{1-6}-alkoxy, optionally substituted C_{1-6}-alkyl, optionally substituted C_{2-6}-alkenyl, optionally substituted C_{2-6}-alkenyloxy, optionally substituted C_{2-6}-alkynyl, optionally substituted C_{2-6}-alkynyloxy, monophosphate, diphosphate, triphosphate, DNA intercalators, photochemically active groups, thermochemically active groups, chelating groups, reporter groups, ligands, carboxy, sulphono, hydroxymethyl, Prot-O-CH_2-, aminomethyl, Prot-N(R^H)-CH_2-, carboxymethyl, sulphonomethyl, where Prot is a protection group for -OH, -SH, and -NH(R^H), respectively, and R^H is selected from hydrogen and C_{1-6}-alkyl; and Q^* is selected from hydrogen, azido, halogen, cyano, nitro, hydroxy, Act-O-, mercapto, Act-S-, C_{1-6}-alkylthio, amino, Act-N(R^H)-,

mono- or di(C_{1-6}-alkyl)amino, optionally substituted C_{1-6}-alkoxy, optionally substituted C_{1-6}-alkyl, optionally substituted C_{2-6}-alkenyl, optionally substituted C_{2-6}-alkenyloxy, optionally substituted C_{2-6}-alkynyl, optionally substituted C_{2-6}-alkynyloxy, DNA intercalators, photochemically active groups, thermochemically active groups, chelating groups, reporter groups, ligands, carboxy, sulphono, where Act is an activation group for -OH, -SH, and -NH(R^H), respectively, and R^H is selected from hydrogen and C_{1-6}-alkyl.

The monomeric L-ribo-LNAs of the general formula II may, as the L-ribo-LNAs incorporated into oligomers, represent various stereoisomers. Thus, the stereochemical variants described above for the L-ribo-LNAs incorporated into oligomers are believed to be equally applicable in the case of monomeric L-ribo-LNAs (however, it should be noted that P should then be replaced with Q).

In a preferred embodiment of the present invention, the monomeric LNA has the general formula IIa



IIa

wherein the substituents are defined as above.

Furthermore, with respect to the definitions of substituents, biradicals, R^*, etc. the same preferred embodiments as defined above for the oligomer according to the invention also apply in the case of monomeric L-ribo-LNAs.

In a particularly interesting embodiment of the monomeric L-ribo-LNAs of the present invention, B designates a nucleobase, preferably a nucleobase selected from thymine, cytosine, uracil, adenine and guanine (in particular adenine and guanine), X is -O-, R^{2*} and R^{4*} together designate a biradical selected from -$(CH_2)_{0-1}$-O-$(CH_2)_{1-3}$-, -$(CH_2)_{0-1}$-S-$(CH_2)_{1-3}$-, and -$(CH_2)_{0-1}$-N(R^N)-$(CH_2)_{1-3}$-, in particular -O-CH_2-, -S-CH_2- and -R^N-CH_2-, where R^N is selected from hydrogen and C_{1-4}-alkyl, Q designates Prot-O-, Q^* designates Act-OH, and R^{1*}, R^2, R^{3*}, R^5, and R^{5*} each designate hydrogen. In this embodiment, R^N may also be selected from DNA intercalators, photochemically active groups, thermochemically active groups, chelating groups, reporter groups and ligands.

In a further particularly interesting embodiment of the monomeric L-ribo-LNAs of the present invention, B designates a nucleobase, preferably a nucleobase selected from thymine, cytosine, uracil, adenine and guanine (in particular adenine and guanine), X is –O–, R^{2*} and R^{4*} together designate a biradical selected from -$(CH_2)_{0-1}$-O-$(CH_2)_{1-3}$-, -$(CH_2)_{0-1}$-S-$(CH_2)_{1-3}$-, and -$(CH_2)_{0-1}$-N(R^N)-$(CH_2)_{1-3}$-, in particular -O-CH_2-, -S-CH_2- and -R^N-CH_2-, where R^N is selected from hydrogen and C_{1-4}-alkyl, Q is selected from hydroxy, mercapto, C_{1-6}-alkylthio, amino, mono- or di(C_{1-6}-alkyl)amino, optionally substituted C_{1-6}-alkoxy, optionally substituted C_{2-6}-alkenyloxy, optionally substituted C_{2-6}-alkynyloxy, monophosphate, diphosphate, and triphosphate, Q^* is selected from hydrogen, azido, halogen, cyano, nitro, hydroxy, mercapto, C_{1-6}-alkylthio, amino, mono- or di(C_{1-6}-alkyl)amino, optionally substituted C_{1-6}-alkoxy, optionally substituted C_{1-6}-alkyl, optionally substituted C_{2-6}-alkenyl, optionally substituted C_{2-6}-alkenyloxy, optionally substituted C_{2-6}-alkynyl, and optionally substituted C_{2-6}-alkynyloxy, R^{3*} is selected from hydrogen, optionally substituted C_{1-6}-alkyl, optionally substituted C_{2-6}-alkenyl, and optionally substituted C_{2-6}-alkynyl, and R^{1*}, R^2, R^5, and R^{5*} each designate hydrogen. Also here, R^N may also be selected from DNA intercalators, photochemically active groups, thermochemically active groups, chelating groups, reporter groups and ligands.

One aspect of the invention is to provide various derivatives of L-ribo-LNAs for solid-phase and/or solution phase incorporation into an oligomer. As an illustrative example, monomers suitable for incorporation of (*1R,3R,4S,7R*)-7-hydroxy-1-hydroxymethyl-3-(thymin-1-yl)-2,5-dioxabicyclo[2.2.1]heptane, (*1R,3R,4S,7R*)-7-hydroxy-1-hydroxymethyl-3-(cytosin-1-yl)-2,5-dioxabicyclo[2.2.1]heptane, (*1R,3R,4S,7R*)-7-hydroxy-1-hydroxymethyl-3-(uracil-1-yl)-2,5-dioxabicyclo[2.2.1]heptane, (*1R,3R,4S,7R*)-7-hydroxy-1-hydroxymethyl-3-(guanin-1-yl)-2,5-dioxabicyclo[2.2.1]heptane, and (*1R,3R,4S,7R*)-7-hydroxy-1-hydroxymethyl-3-(adenin-1-yl)-2,5-dioxabicyclo[2.2.1]heptane using the phosphoramidite approach, the phosphortriester approach, and the *H*-phosphonate approach, respectively, are (*1R,3R,4S,7R*)-7-(2-Cyanoethoxy(diisopropyl-amino) phosphinoxy)-1-(4,4'-dimethoxytrityloxymethyl)-3-(thymin-1-yl)-2,5-dioxabicyclo[2.2.1]heptane, (*1R,3R,4S,7R*)-7-hydroxy-1-(4,4'-dimethoxytrityloxymethyl)-3-(thymin-1-yl)-2,5-dioxabicyclo[2.2.1]heptane-7-*O*-(2-chlorophenylphosphate), and (*1R,3R,4S,7R*)-7-hydroxy-1-(4,4'-dimethoxytrityloxymethyl)-3-(thymin-1-yl)-2,5-dioxabicyclo[2.2.1]heptane-7-*O*-(*H*-phosphonate) and the 3-(cytosin-1-yl), 3-(uracil-1-yl), 3-(adenin-1-yl) and 3-(guanin-1-yl) analogues thereof, respectively. Furthermore, the

analogues where the methyleneoxy biradical of the monomers is substituted with a methylenethio, a methyleneamino, or a 1,2-ethylene biradical are also expected to constitute particularly interesting variants within the present invention. The methylenethio and methyleneamino analogues are believed to equally applicable as the methyleneoxy analogue and therefore the specific reagents corresponding to those mentioned for incorporation of (*1R,3R,4S,7R*)-7-hydroxy-1-hydroxymethyl-3-(thymin-1-yl)-2,5-dioxa-bicyclo[2.2.1]heptane, (*1R,3R,4S,7R*)-7-hydroxy-1-hydroxymethyl-3-(cytosin-1-yl)-2,5-dioxabicyclo[2.2.1]heptane, (*1R,3R,4S,7R*)-7-hydroxy-1-hydroxymethyl-3-(uracil-1-yl)-2,5-dioxabicyclo[2.2.1]heptane, (*1R,3R,4S,7R*)-7-hydroxy-1-hydroxymethyl-3-(guanin-1-yl)-2,5-dioxabicyclo[2.2.1]heptane, and (*1R,3R,4S,7R*)-7-hydroxy-1-hydroxymethyl-3-(adenin-1-yl)-2,5-dioxabicyclo[2.2.1]heptane should also be considered as particularly interesting reactive monomers within the present invention. For the methyleneamine analogue, it should be noted that the secondary amine may carry a substituent selected from optionally substituted C_{1-6}-alkyl such as methyl and benzyl, optionally substituted C_{1-6}-alkylcarbonyl such as trifluoroacetyl, optionally substituted arylcarbonyl and optionally substituted heteroarylcarbonyl.

Also interesting as a separate aspect of the present invention is the variant of formula II or IIa where B is in the "β-configuration".

Preparation of monomers

In a preferred embodiment, α-L-ribo-LNA containing a 2'-*O*,4'-*C*-methylene bridge was synthesised by the following procedure: Benzoylation of 4-*C*-hydroxymethyl-α-D-xylofuranose **1** (T.F. Tam and B. Fraser-Ried, *Can. J. Chem.*, 1979, **57**, 2818) afforded the di-*O*-benzoyl derivative **2** which was subsequently converted into the 1,2-di-*O*-acetylated furanose **3** by acetolysis using 80% acetic acid followed by acetylation. Employing a modified Vorbrüggen methodology (H. Vorbrüggen, K. Krolikiewicz and B. Bennua, *Chem. Ber.*, 1981, **114**, 1234; H. Vorbrüggen and G. Höfle, *Chem. Ber.*, 1981, **114**, 1256), the thymine β-configured nucleoside **4** was stereoselectively obtained by *in situ* silylation of thymine and trimethylsilyl triflate mediated coupling. Treatment of compound **4** with sodium methoxide resulted in deacylation to give nucleoside triol **5**. 4,4'-Dimethoxytrityl protection followed by tosylation afforded the 5'-*O*-4,4'-dimethoxytrityl protected nucleoside derivative **7**. Base-induced ring closure afforded the bicyclic nucleoside derivative **8**. Debenzylation yielded the bicyclic nucleoside analogue **9** which

was transformed into the phosphoramidite derivative **10** for oligonucleotide synthesis. The coupling method used in the example is only one of several possible methods as will be apparent for a person skilled in the art.

As an alternative route the synthetic sequence shown in Figure 3 (Examples 12-14) can be used. Thus, nucleoside **5** was trimesylated to give nucleoside **11** which could be cyclized using NaOH/EtOH/H_2O. Under the experimental conditions used, concomitant conversion of the remaining mesyloxy group to an hydroxyl group was observed yielding nucleoside **12**. Standard DMT-protection as outlined in example 14 is expected to yield nucleoside **8**, a convenient intermediate towards synthesis of the α-L-ribo-LNA nucleoside phosphoramidite derivative **10** (Figure 2).

The described example is meant to be illustrative for the procedures and examples of this invention. The structures of the synthesised compounds were verified using 1D NMR.

The methods depicted in Schemes 1, 2 and 3 can likewise be used to synthesize α-L-ribo-LNA nucleoside derivatives of other pyrimidine bases than thymine, e.g. uracil, cytosine, 5-substituted uracil, 5-substituted cytosine as well as otherwise substituted pyrimidines. Alternatively, the uracil derivatives can be converted to the corresponding cytosine derivatives, and the thymine derivatives to the corresponding 5-methylcytosine derivatives, using well known methods (Koshkin, A. A., Singh, S. K., Nielsen, P., Rajwanshi, V. K., Kumar, R., Meldgaard, M., Olsen, C. E., Wengel, J. *Tetrahedron* **1998**, *54*, 3607; Obika, S., Nanbu, D., Hari, Y., Andoh, J., Morio, K., Doi, T., Imanishi, T. *Tetrahedron Lett.* **1998**, *39*, 5401).

For synthesis of purine α-L-ribo-LNA nucleoside derivatives a number of suitable synthetic methods can be devised. It should be noted that the term "α-face" when mentioned below refers to the α-face of the natural RNA nucleoside monomers, that the term "β-face" when mentioned below refers to the β-face of the natural RNA nucleoside monomers, and that the terms "β-purine nucleoside" or "β-pyrimidine nucleoside" mean that the nucleobases are positioned as in the natural RNA nucleoside monomers. As an example of a possible synthetic route towards the purine α-L-ribo-LNA nucleoside derivatives, cyclization of arabino-configured analogues (2'-OH group positioned at the β-face of the furanose ring) can be utilized. These nucleosides can be prepared from the corresponding arabino-configured parent nucleosides via 5'-oxidation, aldol condensation and reduction.

Protecting group manipulations and activation of the 5'-OH group (positioned at the β-face of the furanose ring) should then prepare for the desired cyclization as mentioned above. Alternatively, 2'-oxidation of the 2'-OH group of 4'-C-hydroxymethyl derivatives of β-purine ribofuranosyl nucleosides (with the 2'-OH and 3'-OH groups positioned at the α-face of the furanose ring and the 3'-OH positioned at the β-face of the furanose ring (or alternatively at the α-face of the furanose ring) with concomitant inversion at C3') followed by stereoselective reduction (using e.g. $NaBH_4$) should furnish the desired nucleoside with inverted configuration at the 2'-carbon atom. Protecting group manipulations and activation of the 5'-OH group (positioned at the β-face of the furanose ring) should then prepare for the desired cyclization as mentioned above. Other procedures can be anticipated to be useful for inversion of the configuration at the 2'-carbon atom of 4'-C-hydroxymethyl derivatives of β-purine ribofuranosyl nucleosides (with the 2'-OH and 3'-OH groups positioned at the α-face of the furanose ring and the 3'-OH group positioned at the β-face of the furanose ring, or alternatively at the α-face with concomitant inversion at C3', of the furanose ring), e.g. the Mitsunobu reaction or nucleophilic displacement reactions of 2'-O-activated derivatives derivatives (e.g., 2'-O-mesyl, 2'-O-tosyl or 2'-O-trifluoromethanesulfonyl derivatives) with *O*-nucleophiles like acetate, benzoate, alkoxide or the like. Subsequent deprotection to give a 5'-hydroxy-4'-C-hydroxymethyl derivative, activation to prepare for cyclization (e.g., by mono- or dimesylation, mono- or ditosylation, or mono- or ditrifluoromethanesulfonation), cyclization (after deprotection of the 2'-OH group if necessary), and deprotections should furnish the desired purine α-L-ribo nucleosides. It should be noted that the purine bases preferably should be protected in the target monomers and that this can be accomplished during the synthetic route of choice, or as the last step, by trimethylsilylation, protection of the free amino group of the purine bases, and desilylation. The starting 4'-C-hydroxymethyl derivatives of β-purine nucleosides may, in one embodiment, be prepared by coupling of furanose derivative 3 (Figure 1) with properly protected adenine or guanine derivatives following the known Vorbrüggen type coupling methods (see e.g. synthesis of nucleoside 4; Figure 1) (Koshkin, A. A., Singh, S. K., Nielsen, P., Rajwanshi, V. K., Kumar, R., Meldgaard, M., Olsen, C. E., Wengel, J. *Tetrahedron* 1998, *54*, 3607).

It is anticipated that inversion of the configuration as described above may be performed on natural β-purine ribofuranosyl nucleosides (with the 2'-OH positioned at the α-face of the furanose ring ring and the 3'-OH group positioned at the β-face of the furanose ring,

or alternatively at the α-face of the furanose ring with concomitant inversion at C3') with the introduction of the additional 4'-C-hydroxymethyl group to follow thereafter using known procedures, e.g. those described above. One may also expect that either enzymatic or chemical transglycosylation reactions on properly derivatized and protected pyrimidine nucleosides, either arabino-configured β-pyrimidine furanosyl nucleosides, arabino-configured 4'-C-hydroxymethyl-β-pyrimidine furanosyl nucleosides, or already cyclized α-L-ribo-LNA pyrimidine nucleosides are possible synthetic routes towards the purine α-L-ribo-LNA nucleoside derivatives. Alternatively, 4-C-hydroxymethylation, inversion of the configuration at the 2-carbon atom, and cyclization, or one of these procedures or two of these procedures (whatever needed depends on the starting material applied) can be performed starting from a furanose or hexose. Subsequently, before or after cyclization, coupling with different bases (purines or pyrimidines - protected if needed) would furnish nucleoside derivatives useful for synthesis of α-L-ribo-LNA pyrimidine and purine nucleosides after the necessary protecting group manipulations and/or OH-group activations. As yet another procedure to synthesize α-L-ribo-LNA pyrimidine or purine nucleosides, direct building-up of the desired nuclebased, in two or more chemical steps) from an appropriately derivatized furanosyl derivative, e.g. furanosyl amine, should be possible.

In a preferred embodiment, the procedures described in examples 15, 16 and 17 (Figure 4) can be used to prepare the purine α-L-LNA monomers, e.g. the adenine or guanine derivatives. Thus, sugar **3** was coupled with N-6-benzoyladenine to give nucleoside **13** which was selectively deacetylated and subsequently converted into the 2'-O-trifluoromethanesulfonyl derivative. Concomitant reaction with potassium acetate gave the 2'-O-acetyl derivative **14** with inversion at C2'. Complete deacylation followed by reprotection of the adenine moiety, selective mesylation of the two primary hydroxyl groups and treatment with sodium hydroxide in water:dioxane afforded the α-L-LNA adenine nucleoside **15**. DMT-protection of nucleoside **15** followed by debenzylation and 3'-O-phosphitylation (Koshkin, A. A., Singh, S. K., Nielsen, P., Rajwanshi, V. K., Kumar, R., Meldgaard, M., Olsen, C. E., Wengel, J. *Tetrahedron* **1998**, *54*, 3607) is one possible route to obtain the phosphoramidite derivative **16**. Debenzylation of **15** followed by selective DMT-protection of the primary hydroxyl group and 3'-O-phosphitylation is another route affording phosphoramidite derivative **16**.

All the methods and procedures described above for synthesis of α-L-ribo-LNA purine nucleosides are also applicable as alternative methods for synthesis of the α-L-ribo-LNA pyrimidine nucleosides.

The methods described above for synthesis of α-L-ribo-LNA pyrimidine and purine nucleosides leads naturally to methods useful for synthesis of 2'-amino and 2'-thio derivatives of α-L-ribo-LNA nucleosides. As one example, cyclization by attack of a 2'-amino or 2'-thio group positioned at the β-face of the furanose ring on a properly activated 5'-OH group should furnish the 2'-amino or 2'-thio α-L-ribo-LNA pyrimidine or purine nucleosides. Alternatively, cyclization by attack of a 5'-amono or 5'-thio group positioned at the β-face of the furanose ring on a properly activated 2'-OH group positioned at the α-face of the furanose ring should furnish the 2'-amino or 2'-thio α-L-ribo-LNA pyrimidine or purine nucleosides. As yet another method, cyclization of properly activated, protected and configured derivatives, e.g. 2'-O,5'-O-dimesyl, 2'-O,5'-O-ditosyl, or 2'-O,5'-O-ditrifluoromethanesulfonyl nucleosides, using amino or thio nucleophiles (e.g. benzylamine and potassium thioacetate, respectively) should furnish the 2'-amino and 2'-thio derivatives of α-L-LNA nucleosides. Likewise, an attack by a 5'-OH group positioned at the β-face of the furanose ring on a properly activated 2'-OH group group positioned at the α-face of the furanose ring should furnish the parent α-L-ribo-LNA pyrimidine or purine nucleosides.

It is expected that the method used for oligomerization of the α-L-ribo-LNA pyrimidine nucleosides mat be used succesfully also for the α-L-ribo-LNA purine nucleosides. Alternatively, any known method for automated or solution-phase synthesis of oligonucleotides and analogues, e.g. the phophortriester method, the H-phosphonate method or any variant of the phosphoramidite method used for oligomerization of the α-L-ribo-LNA pyrimidine nucleosides, should also be applicable.

Preparation of oligomers

Linear-, branched- (M. Grøtli and B. S. Sproat, *J. Chem. Soc., Chem. Commun.*, 1995, 495; R. H. E. Hudson and M. J. Damha, *J. Am. Chem. Soc.*, 1993, **115**, 2119; M. Von Büren, G. V. Petersen, K. Rasmussen, G. Brandenburg, J. Wengel and F. Kirpekar, *Tetrahedron*, 1995, **51**, 8491) and circular- (G. Prakash and E. T. Kool, *J. Am. Chem. Soc.*, 1992, **114**, 3523) oligo- and polynucleotides of the invention may be produced using

the polymerisation techniques of nucleic acid chemistry well known to a person of ordinary skill in the art of organic chemistry. Phosphoramidite chemistry (S. L. Beaucage and R. P. Iyer, *Tetrahedron*, 1993, **49**, 6123; S. L. Beaucage and R. P. Iyer, *Tetrahedron*, 1992, **48**, 2223) was used, but for instance H-phosphonate chemistry, phosphortriester chemistry or enzymatic synthesis could also be used. The standard coupling conditions for the phosphoramidite approach was slightly modified using pyridine hydrochloride instead of 1*H*-tetrazole as a highly efficient reagent for activating nucleoside phosphoramidites during oligonucleotide synthesis, and a prolongation of the coupling time to between 10 to 30 min.

After synthesis of the desired sequence, deprotection and cleavage from the solid support (cleavage from solid support and removal of protection groups using concentrated ammonia in methanol at room temperature for 12 h) and subsequent reversed phase purification using commercially available disposable cartridges (which includes detritylation) yield the final oligomeric product. Alternatively, purification of L-ribo-LNA oligonucleotides can be done using disposable reversed phase HPLC and/or precipitation from ethanol or butanol. Capillary gel electrophoresis was used to verify the purity and the composition of the synthesised oligonucleotide analogues however, purity and composition can also be verified using reversed phase HPLC and MALDI-MS.

Generally, the present invention provides the use of L-ribo-LNAs as defined herein for the preparation of L-ribo-LNA modified oligonucleotides. Is should be understood that L-ribo-LNA modified oligonucleotides may comprise normal nucleosides (i.e. naturally occurring nucleosides such as ribonucleosides and/or deoxyribonucleosides), as well as modified nucleosides different from those defined with the general formula II.

Furthermore, solid support materials having immobilised thereto an optionally nucleobase protected and optionally 5'-OH protected LNA are especially interesting as material for the synthesis of LNA modified oligonucleotides where an LNA monomer is included in at the 3'-end. In this instance, the solid support material is preferable CPG, *e.g.* a readily (commercially) available CPG material onto which a 3'-functionalised, optionally nucleobase protected and optionally 5'-OH protected LNA is linked using the conditions stated by the supplier for that particular material. BioGenex Universial CPG Support (BioGenex, U.S.A.) can e.g. be used. The 5'-OH protecting group may, *e.g.*, be a DMT

group. 3'-functional group should be selected with due regard to the conditions applicable for the CPG material in question.

Applications

The present invention discloses the surprising finding that derivatives of L-ribo-LNAs, when incorporated into partly modified oligonucleotides, decrease the affinity of these modified oligonucleotides for both complementary DNA and RNA compared to the unmodified oligonucleotides. However, when incorporated into fully L-ribo-LNA modified oligonucleotides a dramatically increase in hybridisation properties for both complementary ssDNA and ssRNA is observed. The α-L-ribo-LNA – a special variant of the L-ribo-LNAs – in addition to the described properties has an ability to discriminate between RNA and DNA targets when hybridizing. Depending on the application, the use of fully modified L-ribo-LNA oligonucleotides thus offers the intriguing possibility to either greatly increase the affinity of a standard oligonucleotide without compromising specificity (constant size of oligonucleotide), significantly increase the specificity without compromising affinity (reduction in the size of the oligonucleotide) or specifically hybridize to RNA targets.

It is also believed that L-ribo-LNA modified oligonucleotides, in addition to greatly enhanced hybridisation properties, display many of the useful physicochemical properties of normal DNA and RNA oligonucleotides. The prospect includes excellent solubility, a response of LNA modified oligonucleotides to salts like sodium chloride and tetramethylammonium chloride which mimic that of the unmodified oligonucleotides, the ability of LNA modified oligonucleotides to act as primers for a variety of polymerases, the ability of LNA modified nucleotides to act as primers in a target amplification reaction using a thermostable DNA polymerase, the ability of LNA modified oligonucleotides to act as a substrate for T4 polynucleotide kinase, the ability of biotinylated LNAs to sequence specifically capture PCR amplicons onto a streptavidine coated solid surface, the ability of immobilised LNA modified oligonucleotides to sequence specifically capture amplicons and very importantly the ability of LNA modified oligonucleotides to sequence specifically target double-stranded DNA by strand invasion. Hence, it is apparent to one of ordinary skills in the art that these novel nucleoside analogues are extremely useful tools to improve the performance in general of oligonucleotide based techniques in therapeutics, diagnostics and molecular biology.

An object of the present invention is to provide monomeric L-ribo-LNAs according to the invention which can be incorporated into oligonucleotides using procedures and equipment well known to one skilled in the art of oligonucleotide synthesis.

Another object of the present invention is to provide fully or partly L-ribo-LNA modified oligonucleotides (oligomers) that are able to hybridise in a sequence specific manner to complementary oligonucleotides forming either duplexes or triplexes of substantially higher affinity than the corresponding complexes formed by unmodified oligonucleotides.

Another object of the present invention is to use fully L-ribo-LNA modified oligonucleotides to obtain enhanced specificity of the oligonucleotides without compromising on the affinity.

Another object of the present invention is to provide fully or partly modified oligonucleotides comprising both L-ribo-LNAs, normal nucleosides and other nucleoside analogues.

A further object of the present invention is to exploit the high affinity of L-ribo-LNAs to create fully modified oligonucleotides of extreme affinity that are capable of binding to their target sequences in a dsDNA molecule by way of "strand displacement".

A further object of the invention is to provide different classes of L-ribo-LNAs which, when incorporated into oligonucleotides, differ in their affinity towards their complementary nucleosides. This can be achieved for example by substituting the normal nucleobases G, A, T, C and U with derivatives having, for example, altered hydrogen bonding possibilities.

Another object of the present invention is to provide L-ribo-LNA modified oligonucleotides which are more resistant to nucleases than their unmodified counterparts.

Another object of the present invention is to provide L-ribo-LNA modified oligonucleotides which can discriminate between DNA and RNA targets when hybridizing. It has surprisingly been shown by T_m measurements that the T_m of α-L-ribo-LNA against complementary RNA oligonucleotides is increased 5.7°C per modification compared to only 2.7°C per modification against complementary DNA (as shown in example 11, Table 3). α-L-ribo-LNA oligos will thus have an increased affinity towards RNA compared to

DNA allowing conditions to be created under which α-L-ribo-LNA specifically will hybridize to a given RNA but not to a DNA having the same base sequence. This ability to discriminate between RNA and DNA can be exploited in a number of situations described below.

Another object of the present invention is to provide L-ribo-LNA modified oligonucleotides which can recruit RNAseH.

An additional object of the present invention is to provide L-ribo-LNAs that can act as substrates for DNA and RNA polymerases thereby allowing the analogues to be either incorporated into a growing nucleic acid chain or to act as chain terminators.

A further object of the present invention is to provide L-ribo-LNAs that can act as therapeutic agents. Many examples of therapeutic nucleoside analogues are known and similar derivatives of the nucleoside analogues disclosed herein can be synthesised using the procedures known from the literature (E. De Clercq, *J. Med. Chem.* 1995, **38**, 2491; P. Herdewijn and E. De Clercq: Classical Antiviral Agents and Design of New Antiviral Agents. In: A Textbook of Drug Design and Development; Eds. P. Krogsgaard-Larsen, T. Liljefors and U. Madsen; Harwood Academic Publishers, Amsterdam, 1996, p. 425; I. K. Larsen: Anticancer Agents. In: A Textbook of Drug Design and Development; Eds. P. Krogsgaard-Larsen, T. Liljefors and U. Madsen; Harwood Academic Publishers, Amsterdam, 1996, p. 460).

Double-stranded RNA has been demonstrated to posses anti-viral activity and tumour suppressing activity (Sharp et al., *Eur. J. Biochem.* **230**(1): 97-103, 1995, Lengyel-P. et al., *Proc. Natl. Acad. Sci.* U.S.A., **90**(13): 5893-5, 1993, and Laurent-Crawford et al., *AIDS Res. Hum. Retroviruses*, **8**(2): 285-90, 1992). It is likely that double stranded LNAs may mimic the effect of therapeutically active double stranded RNAs and accordingly such double stranded LNAs have a potential as therapeutic drugs.

When used herein, the term "natural nucleic acid" refers to nucleic acids in the broadest sense, like for instance nucleic acids present in intact cells of any origin or vira or nucleic acids released from such sources by chemical or physical means or nucleic acids derived from such primary sources by way of amplification. The natural nucleic acid may be single, double or partly double stranded, and may be a relatively pure species or a mixture

of different nucleic acids. It may also be a component of a crude biological sample comprising other nucleic acids and other cellular components. On the other hand, the term "synthetic nucleic acids" refers to any nucleic acid produced by chemical synthesis.

The present invention also provides the use of L-ribo-LNA modified oligonucleotides in nucleic acid based therapeutic, diagnostics and molecular biology. The L-ribo-LNA modified oligonucleotides can be used in the detection, identification, capture, characterisation, quantification and fragmentation of natural or synthetic nucleic acids, and as blocking agents for translation and transcription *in vivo* and *in vitro*. In many cases it will be of interest to attach various molecules to L-ribo-LNA modified oligonucleotides. Such molecules may be attached to either end of the oligonucleotide or they may be attached at one or more internal positions. Alternatively, they may be attached to the oligonucleotide via spacers attached to the 5'- or 3'-end. Representative groups of such molecules are DNA intercalators, photochemically active groups, thermochemically active groups, chelating groups, reporter groups, and ligands. Generally all methods for labelling unmodified DNA and RNA oligonucleotides with these molecules can also be used to label L-ribo-LNA modified oligonucleotides. Likewise, all methods used for detecting labelled oligonucleotides generally apply to the corresponding labelled, L-ribo-LNA modified oligonucleotides.

Thus the use of an L-ribo.LNA modified oligonucleotide can be used for the labelling of cells, wherein the label allows the cells to be distinguishable or seperated from unlabelled cells.

Therapy

The term "strand displacement" relates to a process whereby an oligonucleotide binds to its complementary target sequence in a double stranded DNA or RNA so as to displace the other strand from said target strand.

In an aspect of the present invention, L-ribo-LNA modified oligonucleotides capable of performing "strand displacement" are exploited in the development of novel pharmaceutical drugs based on the "antigene" approach. In contrast to oligonucleotides capable of making triple helices, such "strand displacement" oligonucleotides allow any sequence in a dsDNA to be targeted and at physiological ionic strength and pH.

The "strand displacing" oligonucleotides can also be used advantageously in the antisense approach in cases where the RNA target sequence is inaccessible due to intramolecular hydrogen bonds. Such intramolecular structures may occur in mRNAs and can cause significant problems when attempting to "shut down" the translation of the mRNA by the antisense approach.

Other classes of cellular RNAs, like for instance tRNAs, rRNAs, snRNAs and scRNAs, comprise intramolecular structures that are important for their function. These classes of highly structured RNAs do not encode proteins but rather (in the form of RNA/protein particles) participate in a range of cellular functions such as mRNA splicing, polyadenylation, translation, editing, maintenance of chromosome end integrity, etc. Due to their high degree of structure, that impairs or even prevent normal oligonucleotides from hybridising efficiently, these classes of RNAs have so far been difficult to use as antisense targets. However, with the new, surprising results of α-L-ribo-LNA presented herein, targeting these RNAs with the α-L-ribo-LNA is a possibility as described below.

It is known that a number of antibiotics interact with the bacterial ribosome and thereby inhibits translation. Some antibiotics (e.g. streptomycin, tetracycline, spectinomycin, edeine, hygromycin and the neomycins) are known to bind to specific regions in the bacterial 16 S rRNA (Moazed D and Noller HF, *Nature*, 1987, **327**(6121), 389). Similary, other antibiotics (e.g. chloramphenicol, erythromycin, carbomycin and vernamycin B) interacts with specific regions in the bacterial 23 S rRNA (Moazed D and Noller HF, *Biochimie*, 1987, **69**(8), 879). A similar approach seems to be feasible also in higher organisms (Spangler EA and Blackburn EH, *J. Biol. Chem.*, 1985, **260**(10), 6334).

Furthermore, it is known that PNAs – PNAs (Peptide Nucleic Acids) are molecules that interact specifically with DNA in a Watson-Crick base-pairing fashion and do so with a somewhat increased thermal stability (T_m) – targeted to functional and accessible sites in ribosomal RNA can inhibit translation in *Escherichia coli* (Good L and Nielsen PE, *Proc Natl Acad Sci U.S A*, 1998, **95**(5), 2073) indicating that high affinity oligonucleotides which bind to certain sites of rRNA may mimic the effect of rRNA binding antibiotics. Since LNA binds to RNA with an even higher T_m than PNAs do, it is highly likely that LNAs can be designed that specifically binds to bacterial rRNA and inhibits translation in the bacteria. As an extension to this approach it may be possible to exploit the small but significant

differences in the rRNA sequences between higher organisms to design LNA-oligos that inhibits the translation in one, but not in the other. One obvious application of this approach would be to develop LNAs specifically which inhibit translation in Plasmodium spp. (the Malaria parasites), Schistosoma spp. (causing Bilharzia), various filariae (causing Elephantiasis and River Blindness), hookworms (causing anaemia) and other pathogenic parasites.

The use of high affinity L-ribo-LNA monomers should facilitate the construction of antisense probes of sufficient thermostability to hybridise effectively to such target RNAs. Therefore, in a preferred embodiment, L-ribo-LNA is used to confer sufficient affinity to the oligonucleotide to allow it to hybridise to these RNA classes thereby modulating the qualitative and/or quantitative function of the particles in which the RNAs are found.

The L-ribo-LNA modified oligonucleotides to be used in antisense therapeutics are designed with the dual purpose of high affinity and ability to recruit RNAseH. This can be achieved by, for instance, having L-ribo-LNA segments flanking an unmodified central DNA segment. Furthermore, the special ability of the α-L-ribo-LNA to discriminate between RNA and DNA can be exploited in various general therapeutic antisense applications because of the α-L-ribo-LNA's preference for RNA. By designing α-L-ribo-LNA oligonucleotides specific to the RNA of interest unspecific binding to DNA fragments with similar nucleotide sequence as the target RNA is avoided, thereby preventing stable association of the α-L-ribo-LNA oligonucleotides to the chromosomal DNA which can change the structure of the DNA and thus induce mutations in the gene in question. This change in DNA structure and the associated mutations may cause unwanted toxic side-effects.

Yet another embodiment of the present invention is to design ribozymes with increased specificity. Ribozymes are oligodeoxyribonucleotides and analogues thereof which combine the RNAse catalytic activity with the ability of sequence specific interaction with a complementary RNA target. These have attracted much interest as therapeutic molecules and it appears highly likely that the attractive features of α-L-ribo-LNA oligonucleotides can be used to improve the design of ribozymes directed against specific RNAs.

Yet another embodiment of the present invention is L-ribo-LNA oligonucleotides which specifically interact with cellular nucleoproteins which contain RNA as an integrated and

essential component of the active protein, two examples hereof are ribosomes and telomerase. The ability of α-L-ribo-LNA oligonucleotides to inhibit telomerase can be applied to important applications.

The chromosomes of higher eukaryotes (including man) are linear. The primary structure (the DNA sequence) of the chromosome ends has been elucidated and it turns out that the DNA sequences of all chromosome ends – in a particular organism – consist of a simple repeating unit with a protruding single-stranded end. The chromosome end is called the telomere. In man telomeres contain long stretches of double stranded multiple repeats of the sequence 5'-TTAGGG-3' (sequence of one strand, in the direction from the centromere towards the chromosome end). Since all DNA polymerases require both template strand and oligonucleotide primer to initiate the synthesis of a complementary DNA strand, DNA polymerase in it self is not able to replicate the extreme ends of the chromosomes. This would lead to a progressive shortening of the chromosomes, when the chromosomes are replicated. Looking on the length of the telomeres in normal somatic cells the telomer-length indeed seems to become shorter during each cycle of replication until the telomere is only 5-15 kb in length. When the telomeres are that short, cells normally cease to divide and gradually enters the phase of senescence. The only exception to this is the stem-cells. Stem-cells are specialized cells that are able to continue to divide during the life of an organism. Interestingly the telomeres of stem-cells continues to be long (10-15 kb). They do so because of the activity of a particular enzyme, the telomerase. Telomerase is a unique enzyme that is able specifically to prolong the protruding single-stranded end of the telomere, thus allowing the telomere to be stably long. Telomerase is a ribonucleoprotein enzyme, i.e. a protein that contains an RNA and is dependant on the RNA for its enzymatic activity. The structure of telomerase is somewhat similar to reverse transcriptase – a viral protein that also is able to synthesize DNA using an RNA as template.

The enzymatic capacity of telomerase is dependant on the correct positioning of the free telomere 3' end on the RNA molecule to prolong the telomere. Molecules that are able specifically to interact with either the extreme end of the telomere or perhaps with the RNA component of telomerase will inhibit the enzyme. α-L-ribo-LNA can be designed to fulfil these requirements. This will be interesting in e.g. cancer therapy as – except for stem cells – normal somatic cells do not contain detectable telomerase activity which is in vast contrast to cancer cells, most of which contain easily detectable telomerase activity. Cancer cells are immortal, i.e. they do not senesce but continues to proliferate and form

tumour mass until the organism die. The overall evidence to date suggests that the telomerase activity is essential for the immortalization of cancer cells. Interestingly, the telomeres of cancer cells are substantially shorter than the telomeres of stem cells indicating that cancer cells would hit the "telomere length barrier" earlier than stem cells would and suggesting that a drug that specifically inhibits telomerase activity is useful as an anti-cancer drug.

In this view it will be an important issue to exploit the exceptional properties of α-L-ribo-LNA to design short α-L-ribo-LNA-oligomers directed against specific parts of the telomerase RNA component with the purpose to inhibit the telomerase activity of human cancer cells.

Another embodiment of the present invention is the use of L-ribo-LNA oligonucleotides especially α-L-ribo-LNA oligonucleotides as aptamers. This promising new class of therapeutic oligonucleotides are selected *in vitro* to specifically bind to a given target with high affinity, such as for example ligand receptors. Their binding characteristics are likely a reflection of the ability of oligonucleotides to form three dimensional structures held together by intramolecular nucleobase pairing. It is highly likely that aptamers containing α-L-ribo-LNA oligonucleotides may display advantageous characteristics that can be exploited for therapeutic purposes.

In some cases it may be advantageous to down-regulate the expression of a gene whereas in other cases it may be advantageous to activate it. As shown by Møllegaard et al. (Møllegaard, N. E.; Buchardt, O.; Egholm, M.; Nielsen, P. E. *Proc. Natl. Acad. Sci. U.S.A.* 1994, **91**, 3892), oligomers capable of "strand displacement" can function as RNA transcriptional activators. In an aspect of the present invention, the LNAs capable of "strand displacement" are used to activate genes of therapeutic interest.

In chemotherapy of numerous viral infections and cancers, various forms of nucleosides and nucleoside analogues have proven effective. L-ribo-LNA nucleosides are potentially useful as such nucleoside based drugs.

In a number of cases, double-stranded RNA (DS-RNA) has been reported to have specific pharmaceutical activities. Duplexes involving fully L-ribo-LNA modified oligonucleotide(s) are potentially useful as such double-stranded drugs and it is furthermore highly possible

that double-stranded α-L-ribo-LNA oligonucleotides will add important molecules to the repertoire of biologically active double-stranded RNA-like molecules.

The therapeutic potential of double-stranded LNA (DS-LNA) may therefore be in the treatment of cancer or viral infections, as explained below.

Various types of DS-RNAs either alone or in synergy with inteferon-gamma have been reported to inhibit the growth of several types of cancer cells (Borecky et al. *Tex Rep Biol Med*, 1981, **41**, 575; Sharp et al. *Eur J Biochem*, 1995, **230**(1), 97). DS-RNAs inhibit the growth of cancer cells in culture as well as in tumours in experimental animals. At least two double-stranded RNA-activatable enzymes seem to be involved in the tumour-suppressing activity of DS-RNA, the double-stranded RNA-activable protein kinase (PKR) and ribonuclease L (Lengyel-P, *Proc. Natl. Acad .Sci USA*, 1993, **90**(13), 5893). Whereas PKR is activated directly by DS-RNA, RNase L is activated by DS-RNA via (2'-5')oligoadenylate synthetase which is latent unless activated by DS-RNA. DS-RNA also induces natural killer (NK) cell activity and this activity probably contribute to the anti-tumour activity of DS-RNA.

Although naturally occurring DS-RNA typically is associated with virus infection, DS-RNA has been demonstrated to also posses anti-viral activity. DS-RNA has demonstrated its antiviral activity against the human immunodeficiency virus HIV-1 and HIV-2 (Haines et al. *J Cell Biochem*, 1991, **46***(1)*, 9). DS-RNA and thus DS-LNA may therefore be a potential candidate as a therapeutic drug in treating AIDS.

DS-RNA has yet to prove its clinical efficacy in practice. However, mammalian cells contain a number of DS-RNA specific nucleases and perhaps because these activities DS-RNA is rapidly eliminated from patients. LNA is rather similar to RNA and shares most of the chemical characteristics of RNA (Koshkin et al., *Tetrahedron*, 1998, **54**, 3607), LNA form stable duplexes and the structural change from RNA to LNA is rather subtle. Thus, it is likely that adequate double-stranded LNAs may mimic the effect of certain DS-RNAs and accordingly activate PKR and/or (2'-5')oligoadenylate synthetase and since LNA has proven itself to display exonucleolytic stability (Singh et al., *Chem. Commun.*, 1998, 455) it is possible that DS-LNA-molecules may exhibit improved therapeutic efficacy relative to DS-RNA.

The invention also concerns a pharmaceutical composition comprising a pharmaceutically active L-ribo-LNA modified oligonucleotide or a pharmaceutically active L-ribo-LNA monomer as defined above in combination with a pharmaceutically acceptable carrier.

Such compositions may be in a form adapted to oral, parenteral (intravenous, intraperitoneal), intramuscular, rectal, intranasal, dermal, vaginal, buccal, ocularly, or pulmonary administration, preferably in a form adapted to oral administration, and such compositions may be prepared in a manner well-known to the person skilled in the art, e.g. as generally described in "Remington's Pharmaceutical Sciences", 17. Ed. Alfonso R. Gennaro (Ed.), Mark Publishing Company, Easton, PA, U.S.A., 1985 and more recent editions and in the monographs in the "Drugs and the Pharmaceutical Sciences" series, Marcel Dekker.

Diagnostics

Several diagnostic and molecular biology procedures have been developed that utilise panels of different oligonucleotides to simultaneously analyse a target nucleic acid for the presence of a plethora of possible mutations. Typically, the oligonucleotide panels are immobilised in a predetermined pattern on a solid support such that the presence of a particular mutation in the target nucleic acid can be revealed by the position on the solid support where it hybridises. One important prerequisite for the successful use of panels of different oligonucleotides in the analysis of nucleic acids is that they are all specific for their particular target sequence under the single applied hybridisation condition. Since the affinity and specificity of standard oligonucleotides for their complementary target sequences depend heavily on their sequence and size this criteria has been difficult to fulfil so far.

Furthermore, a number of techniques have been developed to characterize the various types of RNA that cells may contain. A common approach to the characterization is nucleic acid hybridisation, examples of such techniques are: *in situ* hybridisation, dot blot hybridisation, reverse dot blot hybridisation, northern hybridisation, and reverse transcription polymerase chain reaction (rtPCR). Often these techniques are prepared on samples containing both DNA and RNA, and frequently this fact creates problems in the assays that easily could be avoided if probes existed that were adequately discriminatory between DNA and RNA. This is in particular a problem in *in situ* hybridisations performed on various tissue specimens. With its highly discriminative hybridization properties towards

RNA an α-L-ribo-LNA oligo can be designed to specifically hybridize with the RNA in the sample thereby eliminating the possibility of erroneous results obtained from hybridization to irrelevant DNAs with the same nucleotide sequence.

In a preferred embodiment, therefore, L-ribo-LNAs can be used as a means to increase affinity and/or specificity of the probes and as a means to equalise the affinity of different oligonucleotides for their complementary sequences. As disclosed herein such affinity modulation can be accomplished by, *e.g.*, replacing selected nucleosides in the oligonucleotide with a L-ribo-LNA carrying a similar nucleobase. In particular, this applies to α-L-ribo-LNA oligonucleotides.

In another preferred embodiment the high affinity and specificity of L-ribo-LNA modified oligonucleotides is exploited in the sequence specific capture and purification of natural or synthetic nucleic acids. In one aspect, the natural or synthetic nucleic acids are contacted with the L-ribo-LNA modified oligonucleotide immobilised on a solid surface. In this case hybridisation and capture occurs simultaneously. The captured nucleic acids may be, for instance, detected, characterised, quantified or amplified directly on the surface by a variety of methods well known in the art or it may be released from the surface, before such characterisation or amplification occurs, by subjecting the immobilised, modified oligonucleotide and captured nucleic acid to dehybridising conditions, such as for example heat or by using buffers of low ionic strength.

The solid support may be chosen from a wide range of polymer materials such as for instance CPG (controlled pore glass), polypropylene, polystyrene, polycarbonate or polyethylene and it may take a variety of forms such as for instance a tube, a micro-titer plate, a stick, a bead, a filter, etc. The L-ribo-LNA modified oligonucleotide may be immobilised to the solid support via its 5' or 3' end (or via the terminus of linkers attached to the 5' or 3' end) by a variety of chemical or photochemical methods usually employed in the immobilisation of oligonucleotides or by non-covalent coupling such as for instance via binding of a biotinylated L-ribo-LNA modified oligonucleotide to immobilised streptavidin. One preferred method for immobilising L-ribo-LNA modified oligonucleotides on different solid supports is photochemical using a photochemically active anthraquinone covalently attached to the 5'- or 3'-end of the modified oligonucleotide (optionally via linkers) as described in (WO 96/31557). Thus, the present invention also provide a surface carrying an LNA modified oligonucleotide.

In another aspect the L-ribo-LNA modified oligonucleotide carries a ligand covalently attached to either the 5'- or 3'-end. In this case the L-ribo-LNA modified oligonucleotide is contacted with the natural or synthetic nucleic acids in solution whereafter the hybrids formed are captured onto a solid support carrying molecules that can specifically bind the ligand.

In still another aspect, L-ribo-LNA modified oligonucleotides capable of performing "strand displacement" are used in the capture of natural and synthetic nucleic acids without prior denaturation. Such modified oligonucleotides are particularly useful in cases where the target sequence is difficult or impossible to access by normal oligonucleotides due to the rapid formation of stable intramolecular structures. Examples of nucleic acids comprising such structures are rRNA, tRNA, snRNA and scRNA.

In another preferred embodiment, L-ribo-LNA modified oligonucleotides designed with the purpose of high specificity are used as primers in the sequencing of nucleic acids and as primers in any of the several well known amplification reactions, such as the PCR reaction. As shown herein, the design of the L-ribo-LNA modified oligonucleotides determines whether it will sustain an exponential or linear target amplification. The products of the amplification reaction can be analysed by a variety of methods applicable to the analysis of amplification products generated with normal DNA primers. In the particular case where the L-ribo-LNA modified oligonucleotide primers are designed to sustain a linear amplification the resulting amplicons will carry single stranded ends that can be targeted by complementary probes without denaturation. Such ends could for instance be used to capture amplicons by other complementary L-ribo-LNA modified oligonucleotides attached to a solid surface.

In another aspect, L-ribo-LNA modified oligos capable of "strand displacement" are used as primers in either linear or exponential amplification reactions. The use of such oligos is expected to enhance overall amplicon yields by effectively competing with amplicon re-hybridisation in the later stages of the amplification reaction. Demers et al. (*Nucl. Acid Res.* 1995, **23**, 3050-3055) discloses the use of high-affinity, non-extendible oligos as a means of increasing the overall yield of a PCR reaction. It is believed that the oligomers elicit these effects by interfering with amplicon re-hybridisation in the later stages of the PCR reaction. It is expected that L-ribo-LNA modified oligos blocked at their 3' end will

provide the same advantage. Blocking of the 3' end can be achieved in numerous ways like for instance by exchanging the 3' hydroxyl group with hydrogen or phosphate. Such 3' blocked L-ribo-LNA modified oligos can also be used to selectively amplify closely related nucleic acid sequences in a way similar to that described by Yu et al. (*Biotechniques*, 1997, **23**, 714-716).

In recent years, novel classes of probes that can be used in for example real-time detection of amplicons generated by target amplification reactions have been invented. One such class of probes have been termed "Molecular Beacons". These probes are synthesised as partly self-complementary oligonucleotides comprising a fluorophor at one end and a quencher molecule at the other end. When free in solution the probe folds up into a hairpin structure (guided by the self-complimentary regions) which positions the quencher in sufficient closeness to the fluorophor to quench its fluorescent signal. Upon hybridisation to its target nucleic acid, the hairpin opens thereby separating the fluorophor and quencher and giving off a fluorescent signal.

Another class of probes has been termed "Taqman probes". These probes also comprise a fluorophor and a quencher molecule. Contrary to the Molecular Beacons, however, the quenchers ability to quench the fluorescent signal from the fluorophor is maintained after hybridisation of the probe to its target sequence. Instead, the fluorescent signal is generated after hybridisation by physical detachment of either the quencher or fluorophor from the probe by the action of the 5´exonuxlease activity of a polymerase which has initiated synthesis from a primer located 5´ to the binding site of the Taqman probe.

High affinity for the target site is an important feature in both types of probes and consequently such probes tends to be fairly large (typically 30 to 40 mers). As a result, significant problems are encountered in the production of high quality probes. In a preferred embodiment, therefore, LNA is used to improve production and subsequent performance of Taqman probes and Molecular Beacons by reducing their size whilst retaining the required affinity.

In a further aspect, L-ribo-LNAs are used to construct new affinity pairs (either fully or partially modified oligonucleotides). The affinity constants can easily be adjusted over a wide range and a vast number of affinity pairs can be designed and synthesised. One part of the affinity pair can be attached to the molecule of interest (*e.g.* proteins, amplicons, en-

zymes, polysaccharides, antibodies, haptens, peptides, PNA, etc.) by standard methods, while the other part of the affinity pair can be attached to *e.g.* a solid support such as beads, membranes, micro-titer plates, sticks, tubes, etc. The solid support may be chosen from a wide range of polymer materials such as for instance polypropylene, polystyrene, polycarbonate or polyethylene. The affinity pairs may be used in selective isolation, purification, capture and detection of a diversity of the target molecules mentioned above.

The principle of capturing a L-ribo-LNA-tagged molecule by ways of interaction with another complementary L-ribo-LNA oligonucleotide (either fully or partially modified) can be used to create an infinite number of novel affinity pairs.

In another preferred embodiment the high affinity and specificity of L-ribo-LNA modified oligonucleotides are exploited in the construction of probes useful in *in-situ* hybridisation. For instance, L-ribo-LNA could be used to reduce the size of traditional DNA probes while maintaining the required affinity thereby increasing the kinetics of the probe and its ability to penetrate the sample specimen.

Purification

Another embodiment of the present invention is to use the L-ribo-LNA oligonucleotides especially α-L-ribo-LNA oligonucleotides in RNA-specific purification procedures. The methods traditionally employed to isolate nucleic acids from prokaryotic cells, eukaryotic cells or from complex biological samples uses organic solvents such as phenol and chloroform. These nucleic acid isolations typically begin with an enzymatic digest of the sample performed with proteases followed by cell lysis using ionic detergents and then extraction with phenol or a phenol/chloroform combination. The organic and aqueous phases are separated and nucleic acids which have partitioned into the aqueous phase are recovered by precipitation with alcohol. However, phenol or a phenol/chloroform mixture is corrosive to human skin and is considered as hazardous waste which must be carefully handled and properly discarded. Additionally, standard extractions using the phenol/chloroform methods result in mixtures of RNA and DNA. Therefore it is advantageous to prepare nucleic acid isolation by exploiting the ability of α-L-ribo-LNA to discriminate between RNA and DNA, thereby obtaining samples of pure RNA.

Kits

The present invention also provides a kit for the isolation, purification, amplification, detection, identification, quantification, or capture of natural or synthetic nucleic acids, where the kit comprises a reaction body and one or more L-ribo-LNA modified oligonucleotides (oligomer) as defined herein. The L-ribo-LNA modified oligonucleotides are preferably immobilised onto said reaction body.

The present invention also provides a kit for the isolation, purification, amplification, detection, identification, quantification, or capture of natural or synthetic nucleic acids, where the kit comprises a reaction body and one or more L-ribo-LNAs as defined herein. The L-ribo-LNAs are preferably immobilised onto said reactions body (e.g. by using the immobilising techniques described above).

For the kits according to the invention, the reaction body is preferably a solid support material, *e.g.* selected from borosilicate glass, soda-lime glass, polystyrene, polycarbonate, polypropylene, polyethylene, polyethyleneglycol terephthalate, polyvinylacetate, polyvinylpyrrolidinone, polymethylmethacrylate and polyvinylchloride, preferably polystyrene and polycarbonate. The reaction body may be in the form of a specimen tube, a vial, a slide, a sheet, a film, a bead, a pellet, a disc, a plate, a ring, a rod, a net, a filter, a tray, a microtitre plate, a stick, or a multi-bladed stick.

The kits are typically accompanied by a written instruction sheet stating the optimal conditions for use of the kit.

EXPERIMENTAL

General

Reactions were conducted under an atmosphere of nitrogen when anhydrous solvents were used. Column chromatography was carried out on glass columns using Silica gel 60 (0.040-0.063 mm). After column chromatography, fractions containing product were pooled, evaporated to dryness under reduced pressure and dried under vacuum to give the product. After drying organic phases using Na_2SO_4, filtration was performed. Petroleum ether of distillation range 60-80 °C was used. Chemical shift values δ are in ppm relative to tetramethylsilane as internal reference (^1H and ^{13}C NMR) and relative to 85% H_3PO_4 (^{31}P NMR). Microanalyses were performed at The Microanalytical Laboratory, Department of Chemistry, University of Copenhagen.

The specific descriptions below are accompanied by Figures 1-4 and Tables 1-3.

Preparation of L-ribo-LNA monomers

Example 1:

5-*O*-Benzoyl-4-*C*-benzoyloxymethyl-3-*O*-benzyl-1,2-*O*-isopropylidene-α-D-glucofuranose (2).

To a stirred ice cold solution of 3-*O*-benzyl-4-*C*-hydroxymethyl-1,2-isopropylidene-α-D-glucofuranose (**1**) (5.00 g, 0.016 mol) in anhydrous pyridine (60 cm^3) was added benzoyl chloride (4.1 cm^3, 0.035 mol). After stirring at room temperature for 4 h, the reaction mixture was cooled to 0 °C, H_2O (50 cm^3) was added, and the mixture was extracted with dichloromethane (100 cm^3 x 3). The combined organic phase was washed with saturated aqueous solutions of sodium hydrogen carbonate (30 cm^3 x 3) and brine (20 cm^3 x 3), dried (Na_2SO_4) and evaporated to dryness under reduced pressure. The residue was purified by silica gel column chromatography using first petroleum ether/dichloromethane (1:1, v/v) and then dichloromethane/methanol (99:1, v/v) as eluent to give furanose **2** (7.50 g, 90%) as a yellowish oil after evaporation of the solvents under reduced pressure. δ_H ($CDCl_3$) 8.02-7.23 (15H, m), 6.08 (1H, d, *J* 4.2), 4.81-4.50 (7H, m), 4.22 (1H, d, *J* 1.0), 1.59 (3H, s), 1.37 (3H, s). δ_C ($CDCl_3$) 166.1, 165.8, 136.7, 133.1, 133.0, 129.9, 129.7, 129.6, 129.5, 128.5, 128.4, 128.3, 128.0, 127.9, 113.3, 105.4, 86.4, 85.1, 83.8, 72.3, 64.3, 63.8,

27.0, 26.4. FAB-MS *m/z* 521 $[M+H]^+$. Found (%) C, 69.1; H, 5.9; $C_{30}H_{32}O_8$ requires C, 69.2; H, 6.2.

Example 2:

5-*O*-Benzoyl-4-*C*-benzoyloxymethyl-3-*O*-benzyl-1,2-di-*O*-acetyl-D-glucofuranose (3). A solution of furanose **2** (7.40 g, 0.014 mol) in 80% acetic acid (60 cm^3) was stirred 9 h at 90 °C. The mixture was evaporated to dryness under reduced pressure and the residue was coevaporated with toluene (10 cm^3 x 3) and dissolved in anhydrous pyridine (80 cm^3). Acetic anhydride (5.5 cm^3) was added and the solution was stirred for 46 h at room temperature. The mixture was evaporated to dryness under reduced pressure and the residue was coevaporated with toluene (10 cm^3 x 3) and dissolved in dichloromethane (150 cm^3). The solution was washed with saturated aqueous solutions of sodium hydrogen carbonate (30 cm^3 x 3) and brine (30 cm^3 x 3), dried (Na_2SO_4) and concentrated under reduced pressure. The residue was purified by silica gel column chromatography using first petroleum ether/dichloromethane (1:1, v/v) and then dichloromethane/methanol (99:1, v/v) as eluent to give the anomeric mixture **3** (α:β = 3:1, 7.33 g, 92%) as a clear oil after evaporation of the solvents under reduced pressure. This oil was used in the next step without further purification. δ_C ($CDCl_3$) 169.4, 169.0, 165.8, 165.6, 137.0, 133.2, 133.1, 133.0, 129.6, 129.5, 129.2, 128.3, 127.8, 127.7, 127.4, 99.4, 92.3, 87.0, 83.2, 82.2, 80.7, 77.4, 76.9, 76.3, 73.2, 72.4, 20.9, 20.8, 20.6, 20.3. FAB-MS *m/z* 562 $[M]^+$.

Example 3:

1-(2-*O*-Acetyl-5-*O*-benzoyl-4-*C*-benzoyloxymethyl-3-*O*-benzyl-β-D-xylofuranosyl)thymine (4).

To a stirred suspension of the anomeric mixture **3** (7.26 g, 0.013 mol) and thymine (3.25 g, 0.028 mol) in anhydrous acetonitrile (80 cm^3) was added *N,O*-bis(trimethylsilyl)acetamide (19.1 cm^3, 0.077mol). The reaction mixture was stirred at 60 °C for 1 h and then cooled to 0 °C. Trimethylsilyl triflate (4.1 cm^3, 0.023 mol) was added drop-wise during 10 min and the mixture was subsequently heated for 22 h under reflux. After cooling to room temperature, a saturated aqueous solution of sodium hydrogen carbonate (30 cm^3) was added and extraction was performed using dichloromethane (100 cm^3 x 3). The combined organic phase was washed with saturated aqueous solutions of sodium hydrogen carbonate (30 cm^3 x 3) and brine (50 cm^3 x 3), dried (Na_2SO_4) and concentrated under reduced pressure. The residue was purified by silica gel column chromatography using dichloromethane/ methanol (0.5-2.0% methanol, v/v) as eluent to give nucleoside **4** (6.88 g, 85 %) as a white

solid material after evaporation of the solvents under reduced pressure. δ_H ($CDCl_3$) 8.97 (1H, br s), 8.04-7.23 (16H, m), 6.37 (1H, d, *J* 3.6), 5.42 (1H, t, *J* 3.1), 4.89-4.56 (6H, m), 4.22 (1H, d, *J* 2.6), 2.13 (3H, s), 1.74 (1H, d, *J* 0.8). δ_C ($CDCl_3$) 169.9, 166.0, 165.7, 163.4, 150.4, 136.2, 135.2, 133.5, 133.4, 129.8, 129.7, 129.6, 129.5, 129.0, 128.6, 128.4, 128.2, 112.0, 87.4, 86.0, 81.3, 80.3, 72.6, 63.1, 62.9, 20.8, 12.3. FAB-MS *m/z* 629 $[M+H]^+$. Found (%) C, 64.4; H, 4.9; N, 4.4; $C_{34}H_{32}N_2O_{10}0.25H_2O$ requires C, 64.5; H, 5.1; N, 4.4.

Example 4:

1-(3-*O*-Benzyl-4-*C*-hydroxymethyl-β-D-xylofuranosyl)thymine (5).

To a stirred solution of nucleoside **4** (9.00 g, 0.014 mol) in methanol (130 cm^3) was added sodium methoxide (3.87 g, 0.0716 mol). The reaction mixture was stirred at room temperature for 4 h and then neutralized with dilute hydrochloric acid. The mixture was evaporated to dryness under reduced pressure followed by coevaporation using toulene (15 cm^3 x 3). The residue was purified by silica gel column chromatography using dichloro-methane/methanol (4-15% methanol, v/v) as eluent to give nucleoside triol **5** (4.82 g, 89%) as a white solid material after evaporation of the solvents under reduced pressure. δ_H (CD_3OD) 7.89 (1H, d, *J* 1.2), 7.40-7.24 (5H, m), 5.97 (1H, d, *J* 6.2), 4.83-4.65 (2H, m), 4.53 (1H, t, *J* 6.2), 4.21 (1H, d, *J* 6.2), 3.84 (1H, d, *J* 12.0), 3.63 (1 H, d, *J* 12.0), 3.59 (2H, d, *J* 2.6), 1.82 (1H, d, *J* 1.1). δ_C (CD_3OD) 164.4, 150.9, 137.5, 136.6, 127.5, 127.0, 126.9, 109.8, 86.7, 86.4, 82.8, 78.0, 72.1, 62.3, 61.1, 10.5 (CH_3). FAB-MS *m/z* 379 $[M+H]^+$. Found (%) C, 56.2; H, 6.0; N, 7.0; $C_{18}H_{22}N_2O_7,0.25H_2O$ requires C, 56.5; H, 5.9; N, 7.3.

Example 5:

1-(3-*O*-Benzyl-4-*C*-(4,4'-dimethoxytrityloxymethyl)-β-D-xylofuranosyl)thymine (6).

To a solution of nucleoside 1-(3-*O*-Benzyl-4-*C*-hydroxymethyl-β-D-xylofuranosyl)thymine **5** (5.38 g, 14.2 mmol) in anhydrous tetrahydrofuran (400 cm^3) was added $AgNO_3$ (2.66 g, 15.7 mmol) followed by anhydrous pyridine (5.7 cm^3) and 4,4'-dimethoxytrityl chloride (5.30 g, 15.6 mmol). The mixture was stirred in the dark under nitrogen for 18 h at room temperature. The reaction was quenched by addition of a saturated aqueous solution of sodium hydrogen carbonate (10 cm^3) and the resulting mixture was extracted with dichloromethane. The combined organic phase was evaporated to dryness under reduced pressure and the residue was co-evaporated with toluene and was purified by silica gel column chromatography using dichloromethane/methanol/pyridine (0.5 % methanol; 0.5 % pyridine, v/v) as eluent to afford nucleoside **6** (3.13 g, 31%) as a white foam after evaporation of the solvents. δ_c ($(CD_3)_2SO$) 164.1 (C-4), 158.4, 145.1, 138.5, 137.0, 135.9,

135.7, 130.1, 130.1, 129.2, 128.5, 128.5, 128.2, 128.1, 127.7, 127.6, 127.0, 125.7,113.5 (DMT, benzyl, C-6), 151.4 (C-2), 110.1 (C-5), 85.8, 85.2, 84.6, 83.5 (C-1', C-3', C-4', DMT), 76.8 (C-2'), 72.3 (CH_2Ph), 65.2 (C-5''), 62.1 (C-5'), 55.4 (2x CH_3O), 12.6 (5-CH_3).

Example 6:

1-(3-*O*-Benzyl-4-*C*-(4,4'-dimethoxytrityloxymethyl)-2,5-di-*O*-(*p*-toluenesulphonyl)-β-D-xylofuranosyl)thymine (7).

To a solution of nucleoside **6** (2.79 g, 3.9 mmol) in anhydrous pyridine (50 cm^3) was added a catalytic amount of 4-(*N*,*N*-dimethylamino)pyridine and *p*-toluenesulphonyl chloride (6.50 g, 34 mmol). The mixture was stirred in the dark for 24 h at room temperature under nitrogen. The reaction was quenched by addition of a saturated aqueous solution of sodium hydrogen carbonate (100 cm^3) and the resulting mixture was extracted with dichloromethane. The combined organic phase was washed with saturated aqueous solutions of sodium hydrogen carbonate (3 x 75 cm^3) and sodium chloride (2 x 75 cm^3). The separated organic phase was dried (Na_2SO_4) and evaporated to dryness under reduced pressure. The residue was purified by silica gel column chromatography using dichloromethane/methanol/pyridine (0.5 % methanol; 0.5 % pyridine, v/v) as eluent to afford nucleoside **7** (2.40 g, 62 %) as a yellowish foam after evaporation of the solvents. δ_C ($(CD_3)_2SO$) 163.2 (C-4), 158.2, 145.9, 145.1,144.3, 136.8, 135.0, 134.9, 134.8, 131.8, 131.6, 130.2, 130.0, 129.7, 128.2, 127.9, 127.8, 127.6, 127.5, 127.5, 127.4, 126.8, 113.3 (DMT, C-6, 2 x Ts, benzyl), 150.2 (C-2), 110.8 (C-5), 95.0, 86.2 (DMT, C-4'), 82.2, 81.9 (C-1', C-2'), 81.2 (C-3'), 72.9 (CH_2Ph), 79 (C-5''), 64 (C-5'), 55.1 (2 x CH_3O), 21.2, 21.2 (2 x CH_3), 12.0 (5-CH_3).

Example 7:

(1R,3*R*,4*S*,7*R*)-7-Benzyloxy-1-(4,4'-dimethoxytrityloxymethyl)-3-(thymin-1-yl)-2,5-dioxabicyclo[2.2.1]heptane (8).

To a solution of nucleoside **7** (3.87 g, 3.92 mmol) in a mixture of ethanol and H_2O (1:1, v/v) was added an aqueous solution of NaOH (2M, 8 cm^3). The mixture was heated under reflux for 24 h and after cooling extracted with dichloromethane. The combined organic phase was washed with a saturated aqueous solution of sodium hydrogen carbonate (2 x 75 cm^3) and evaporated to dryness under reduced pressure. The residue was purified by silica gel column chromatography using dichloromethane/methanol/pyridine (0.5 % methanol; 0.5 % pyridine, v/v) as eluent to afford nucleoside **8** (2.10 g, 81 %) as a white foam after evaporation of the solvents. δ_C ($(CD_3)_2SO$) 163.8 (C-4), 158.2, 158.1, 144.7,

137.7, 135.9, 135.2, 135.1, 129.8, 129.7, 128.3, 127.9, 127.7, 127.7, 127.4, 126.7, 113.35 (DMT, benzyl, C-6) 150.3 (C-2), 108.1 (C-5), 88.4, 85.5 (C-4', DMT), 86.4 (C-1'), 79.5 (C-2'), 76.3 (C-3'), 72.6 (C-5'), 71.2 (CH_2Ph), 58.9 (C-5''), 55.1 (2 x CH_3O), 12.4 (5-CH_3).

Example 8:

(1R,3*R*,4*S*,7*R*)-1-(4,4'-Dimethoxytrityloxymethyl)-7-hydroxy-3-(thymin-1-yl)-2,5-dioxabicyclo[2.2.1]heptane (9).

To a solution of nucleoside **8** (1.09 g, 1.65 mmol) in methanol (30 cm^3) was added ammonium formate (0.33 g, 5.29 mmol). A catalytic amount of Pd/C suspended in methanol (10 cm^3) was added and the mixture was heated for 2 h under reflux. After cooling to room temperature, the mixture was evaporated to dryness under reduced pressure and the residue was purified by silica gel column chromatography using dichloromethane/methanol/pyridine (2 % methanol; 0.5 % pyridine, v/v) as eluent to afford nucleoside **9** (0.76 g, 80 %) as a white solid material after evaporation of the solvents. δ_C ($(CD_3)_2SO$) 163.9 (C-4), 158.2, 144.8, 135.8, 135.4, 135.3, 129.8, 127.9, 127.7, 126.8, 113.3 (DMT, C-6), 150.4 (C-2), 108.0 (C-5), 89.2, 85.4 (C-4', DMT), 86.4 (C-1'), 78.9 (C-2'), 72.9 (C-3'), 72.3 (C-5'), 59.9 (C-5''), 55.1 (2 x CH_3O), 12.5 (5-CH_3).

Example 9:

(1R,3*R*,4*S*,7*R*)-7-(2-Cyanoethoxy(diisopropylamino)phosphinoxy)-1-(4,4'-dimethoxytrityloxymethyl)-3-(thymin-1-yl)-2,5-dioxabicyclo[2.2.1]heptane (10).

To a solution of nucleoside **9** (420 mg, 0.73 mmol) in anhydrous dichloromethane (4 cm^3) was added *N,N*-diisopropylethylamine (0.4 cm^3) and 2-cyanoethyl *N,N*-diisopropyl-phosphoramidochloridite (0.4 cm^3). The mixture was stirred in the dark under nitrogen for 18 h at room temperature. The reaction was quenched by addition of methanol and the mixture was diluted with ethyl acetate (10 cm^3), washed with saturated aqueous solutions of sodium hydrogen carbonate (3 x 10 cm^3) and sodium chloride (2 x 10 cm^3) and was evaporated to dryness under reduced pressure. The residue was co-evaporated with anhydrous acetonitrile and was purified by silica gel column chromatography using petroleum ether/ethyl acetate/pyridine (30 – 40 % ethyl acetate; 0.2 % pyridine, v/v) as eluent to give an oli. This oil was dissolved in dichloromethane (1 cm^3) and a product was precipitated from petroleum ether (20 cm^3) at – 40 °C with vigorous stirring. The precipitate was collected by filtration and co-evaporated with anhydrous acetonitrile to give nucleoside **10** (117 mg, 21 %) as a white foam. δ_P (CH_3CN) 149.9, 149.3.

Preparation of LNA oligonucleotides

Example 10:

Synthesis of unmodified oligonucleotides and oligonucleotides comprising L-ribo-LNA derived from phosphoramidite 10 (formula X).

L-ribo-LNA and reference oligonucleotides were prepared on a Biosearch 8750 DNA Synthesizer. Coupling of amidite **10** was performed by "hand coupling" (premixing amidite and the activator in acetonitrile in a syringe; then flushing the column reactor approximately twice every minute throughout the coupling time applied; CPG solid supports). Synthesis of the L-ribo-LNAs were accomplished using pyridine hydrochloride as activator (10-30 min coupling time; step-wise coupling yields for amidite **10** were 96-99%). The unmodified 2'-deoxynucleoside 2-cyanoethyl *N,N*-diisopropylphosphoramidites were coupled by use of the standard DNA-program of the synthesiser except for the couplings immediately following an **X** monomer which were conducted according to the RNA program of the synthesiser. After completion of the sequences, deprotection using concentrated ammonia in methanol (32% (w/w), room temperature, 12 h) of 5'-*O*-DMT-ON oligos and subsequently reversed phase purification (commercially available disposable cartridges (Cruachem); procedure includes detritylation) yielded the final oligomeric products. However, for the unmodified oligonucleotides and the L-ribo-LNA comprising only one **X** monomer the 5'-*O*-DMT group was removed on the synthesiser immediately after completion of the sequences. Subsequent treatment with concentrated ammonia in methanol (32% (w/w), 12 h, 55 °C) and ethanol precipitation afforded the product oligomers. Capillary gel electrophoresis was used to analyse the purity of the synthesised L-ribo-LNAs.

Hybridisation data

Example 11:

Thermostability of oligonucleotides comprising monomer X.

The thermostability of the L-ribo-LNA modified oligonucleotides were determined spectrophotometrically using a spectrophotometer equipped with a thermoregulated Peltier element. Hybridisation mixtures of 1 ml were prepared using a medium salt buffer solution (10mM Na_2HPO_4, pH 7.0, 100mM NaCl, 0.1mM EDTA) and equimolar (1 μM or 1.5 μM) amounts of the different L-ribo-LNA modified oligonucleotides and their complementary DNA or RNA oligonucleotides. Identical hybridisation mixtures using the

unmodified oligonucleotides were prepared as references. The absorbance at 260 nm was recorded while the temperature was raised linearly from 10-90 °C (1°C/min). The melting temperatures (T_m values) were obtained as the maxima (+/- 1 °C) of first derivative of the melting curves. Tables 1-3 summarise the results (L-ribo-LNAs are marked with bold). Figure 2 illustrates the monomeric L-ribo-LNA used.

From Table 1 it can be seen that incorporation of one or more consecutive α-L-ribo-LNA monomers **X** into an oligonucleotide sequence (**A**) and (**B**), does not change the binding affinity of the α-L-ribo-LNAs toward complementary DNA, while the binding affinity towards complementary RNA is strongly increased.

Table 2 shows binding studies of homo-tyhmine diastereoisomeric LNAs towards RNA (rA_{14}), singly mis-matched RNA (5'-r(A_6CA_7)), enantiomeric RNA (*ent*-rA_{14}) and singly mis-matched enantiomeric RNA (*ent*-5'-r(A_6CA_7)).

Table 3 shows binding studies of mixed-sequence 9-mer DNA, LNA and α-L-ribo-LNA.

Alternative method

Example 12:

1-(3-*O*-Benzyl-2,5-di-*O*-methanesulfonyl-4-*C*-(methanesulfonyloxymethyl)-β-D-xylofuranosyl)thymine (11). To a solution of nucleoside **5** (1100 mg, 2.91 mmol) in anhydrous tetrahydrofuran (20 cm^3) was added anhydrous pyridine (5 cm^3) followed by methanesulfonyl chloride (1.2 ml, 15.5 mmol). The mixture was stirred under a nitrogen atmosphere for 18 h at room temperature. The reaction mixture was evaporated to dryness under reduced pressure and dissolved in ethyl acetate. The organic phase was washed with saturated aqueous solution of sodium hydrogen carbonate (3 x 10 cm^3) and dried (Na_2SO_4). The organic phase was evaporated to dryness under reduced pressure. The residue was purified by silica gel column chromatography using dichloromethane/ methanol (2 % methanol, v/v) as eluent to afford nucleoside **11** (908 mg, 51 %). δ_c ($CDCl_3$) 163.3, 150.6, 135.6, 134.6, 128.7, 128.3, 112.2, 87.9, 85.0, 83.1, 80.9, 77.2, 76.9, 76.6, 73.3, 66.6, 66.2, 38.6, 37.6, 37.6, 12.2.

Example 13:

(1*R*,3*R*,4*S*,7*R*)-1-(Hydroxymethyl)-7-benzyloxy-3-(thymin-1-yl)-2,5-dioxabicyclo-[2.2.1]heptane (12).

To a solution of nucleoside **11** (329 mg, 0.54 mmol) in ethanol/water (10 cm^3, 1:1, v/v) was added 6M NaOH (aq) (0.9 ml, 5.4 mmol). The mixture was refluxed at 80 °C for 43 h followed by evaporation to dryness under reduced pressure. The residue was purified by silica gel column chromatography using dichloromethane/methanol (2.4 % methanol, v/v) as eluent to afford nucleoside **12** (85 mg, 44 %). δ_c ($(CD_3)_2SO$) 163.8, 150.3, 138.0, 135.8, 128.3, 127.7, 127.5, 108.0, 90.2, 86.5, 86.4, 79.3, 76.5, 72.5, 71.2, 57.2, 40.2, 40.0, 39.8, 39.6, 39.4, 39.2, 39.0, 12.3.

Example 14:

Synthesis of nucleoside 8 from nucleoside 12.

Standard DMT-protection of the primary hydroxy group of nucleoside **12** (e.g. using the same procedure as for preparation of nucleoside **6** by DMT-protection of the primary hydroxy group of nucleoside **5**) would give nucleoside **8** which can be used in the synthesis of α-L-ribo-LNA nucleoside phosphoramidite derivative **10** (see Figure 2 and the relevant examples).

Example 15:

9-(2-*O*-Acetyl-5-*O*-benzoyl-4-*C*-(benzoyloxymethyl)-3-*O*-benzyl-α-L-ribofuranosyl)-6-*N*-benzoyladenine (14).

Sugar **3** (2.05 g) was dissolved in anhydrous acetonitrile (30 mL). *N*-6-Benzoyladenine (1.86 g) followed by $SnCl_4$ (1.3 mL) were added and the resulting mixture was stirred at room temperature for 3.7 h whereupon a saturated aqueous solution of $NaHCO_3$ was added until neutralization. After filtration through Celite, the filtrate was washed successively with a saturated aqueous solution of $NaHCO_3$ (3 x 150 mL) and H_2O (2 x 150 mL), dried (Na_2SO_4) and evaporated to dryness under reduced pressure. The residue was purified by silica gel column chromatography (40-60% NaOAc in petroleum ether) to give a fully protected nucleoside intermediate (1.40 g, 52% yield). This intermediate (1.29 g) was dissolved in methanol (35 mL) and a saturated solution of NH_3 in methanol (35 mL) was added. After stirring at 0 °C for 2.3 h, the mixture was evaporated to dryness under reduced pressure and the residue was purified by by silica gel column chromatography (1% methanol in dichloromethane) to give an intermediate which was dissolved in anhydrous dichloromethane (40 mL). After cooling to -50 °C, anhydrous

pyridine (3 mL) was added together with trifluoromethanesulfonic anhydride (0.65 mL). After stirring for 50 min, additional trifluoromethanesulfonic anhydride (0.65 mL) was added and stirring was continued at -10 °C for 1 h. Dichloromethane (100 mL) was added and washing was performed using a saturated aqueous solution of $NaHCO_3$ (3 x 100 mL). The separated organic phase was dried (Na_2SO_4) and evaporated to dryness under reduced pressure to give an intermediate. This intermediate was dissolved in toluene (20 mL) and KOAc (0.85 g) and 18-crown-6 (0.92 g) were added and the resulting mixture was stirred at 80 °C for 7 h whereupon evaporation to dryness under reduced pressure afforded a residue which was purified by silica gel column chromatography (0-1.5% methanol in dichloromethane) to give nucleoside **14** (1.1 g, 84% for three steps). δ_C ($CDCl_3$) 168.8, 165.8, 142.7, 136.0, 133.5, 133.3, 132.7, 129.6, 129.6, 128.8, 128.6, 128.5, 128.4, 128.4, 128.1, 127.8, 83.8, 82.2, 78.4, 74.3, 70.8, 64.7, 63.4, 20.5. MS (*m/z*) 742.0 $[M+H]^+$.

Example 16:

(1*R*,3*R*,4*S*,7*R*)-7-Benzyloxy-1-hydroxymethyl-3-(*N*-6-benzoyladenin-9-yl)-2,5-dioxabicyclo[2.2.1]heptane (15).

Nucleoside **14** (3.05 g) was dissolved in a saturated solution of NH_3 in methanol (200 mL) and stirred at room temperature for 4 days whereupon a 33% aqueous solution of NH_3 (60 mL) was added and stirring was continued for 4 h. The mixture was evaporated to dryness under reduced pressure to give an intermediate which was dissolved in anhydrous pyridine (100 mL). TMSCl (7.8 mL) was added and stirring was continued at room temperature for 5 h. After cooling to 0 °C, benzoyl chloride (2.4 mL) was added and stirring was continued at room temperature for 16 h. H_2O (50 mL) was added followed after 5 min by a 25% saturated aqueous solution of NH_3 (25 mL). After stirring for 20 min at room temperature, the mixture was evaporated to dryness under reduced pressure and the residue was purified by silica gel column chromatography (2-5% methanol in dichloromethane) to give an intermediate (1.76 g, 87% over two steps). This intermediate (326 mg) was dissolved in anhydrous pyridine (50 mL) and mesyl chloride (0.11 mL) was added at 0 °C under stirring. After stirring for 2 h, H_2O (5 mL) was added and the volume of the mixture was reduced to approximately 50% by evaporation under reduced pressure. Dichloromethane (100 mL) was added, and washing was performed with a saturated aqueous solution of $NaHCO_3$ (3 x 20 mL). The organic phase was dried (Na_2SO_4) and evaporated to dryness under reduced pressure. The residue was purified by silica gel column chromatography (2-4% methanol in dichloromethane) to give an

intermediate (284 mg). This intermediate (354 mg) was dissolved in a mixture of dioxane (15 mL), H_2O (15 mL) and 2 M NaOH (5.5 mL). After stirring for 72 h under reflux, a 7% (w/w) solution of HCl in dioxane was added until neutralization. Washing was performed with a saturated aqueous solution of $NaHCO_3$ (2 x 100 mL) and the organic phase was dried (Na_2SO_4) and evaporated to dryness under reduced pressure. The residue was purified by silica gel column chromatography (0-4% methanol in dichloromethane) to give the bicyclic nucleoside **15** (24 mg). δ_C ($(CD_3)_2SO$) 156.0, 152.6, 149.4, 138.8, 138.0, 128.3, 127.7, 127.5, 118.3, 89.7, 83.9, 79.7, 77.0, 73.0, 71.2, 57.2. δ_H ($(CD_3)_2SO$) 8.38 (1H, s), 8.14 (1H, s), 7.40-7.30 (7H, m), 6.37 (1H, s), 5.06 (1H, t, *J* 5.8 Hz), 4.73-4.66 (3H, m), 4.46 (1H, s), 4.15 (1H, d, *J* 8.4 Hz), 4.04 (1H, d, *J* 8.2 Hz), 3.75 (2H, d, *J* 5.7 Hz).

Example 17:

(1*S*,3*R*,4*S*,7*R*)-7-(2-Cyanoethoxy(diisopropylamino)phosphinoxy)-1-(4,4'-dimethoxytrityloxymethyl)-3-(6-*N*-benzoyladenin-9-yl)-2,5-dioxabicyclo[2.2.1]heptane (16).

DMT-protection of nucleoside **15** followed by debenzylation and 3'-*O*-phosphitylation is expected to furnish phosphoramidite derivative **16**. Another possible route affording **16** from nucleoside **15** is debenzylation of **15** followed by selective DMT-protection of the primary hydroxyl group and eventual 3'-*O*-phosphitylation. The reactions outlined in this example follows standard procedures (See, e.g.: Koshkin, A. A., Singh, S. K., Nielsen, P., Rajwanshi, V. K., Kumar, R., Meldgaard, M., Olsen, C. E., Wengel, J. *Tetrahedron* **1998**, *54*, 3607).

Table 1:

Sequence[a]		T_m (°C)[b]	T_m (°C)[c]
5'-T_7**X**T_6	**(A)**	32	33
5'-T_5**X**$_4T_5$	**(C)**	36	46
5'-T_3(**Y**)$_4$(**X**)$_4T_3$	**(F)**	64	63
5'-**X**$_9$**T**	**(G)**	63	66
5'-T_{10}	**(E')**	24/20	18
5'-T_{14}	**(E)**	32	28

[a] **X** = monomer derived from phosphoramidite **10**

Y = LNA monomers containing a 2'-*O*,4'-*C*-methylene bridge, cf. Singh et al. *(above)*

[b]Complexed with 5'-dA_{14}

[c]Complexed with 5'-rA_{14}

Table 2:

Sequence[a]	rA_{14} T_m (°C)	5'-r(A_6CA_7) T_m (°C)	*ent*-rA_{14} T_m (°C)	*ent*-5'-r(A_6CA_7) T_m (°C)
T_{10}	18	no T_m[c]	no T_m[c]	no T_m[c]
5'-(**Y**)$_9$**T**	71	61	52	51
5'-(**X**)$_9$**T**	66	49	39	no T_m[c]
5'-(*xylo*-**Y**)$_9$**T**	57	47	39	36
5'-(*xylo*-**X**)$_9$**T**	no T_m[d]	no T_m[d]	no T_m[d]	no T_m[d]

[a] as above for Table 1;

[d] no co-operative melting point T_m was measured in the temperature range 10 - 95 °C

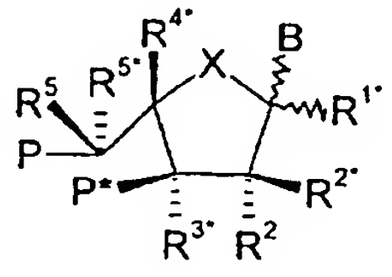
Table 3:

	5'-d(GZGA**Z**AZGC) vs:					
	3'-d(CACT**N**TACG)				3'-r(CACU**N**UACG)	
N =	A	C	T	G	A	C
Entry	T_m (°C)	T_m (°C)	T_m (°C)	T_m (°C)	T_m (°C)	T_m (°C)
1 **Z** = T	28/28*	11/13*	12/15*	19/20*	28/29*	10/no T_m*
2 **Z** = **Y**	44	23	27	30	50	33
3 **Z** = **X**	37	19	19	28	45	23

* results of two identical experiments

CLAIMS

1. An oligomer comprising at least one nucleoside analogue of the general formula I



I

wherein X is selected from -O-, -S-, -N(R^{N*})-, -C(R^6R^{6*})-;

B is selected from hydrogen, hydroxy, optionally substituted C_{1-4}-alkoxy, optionally substituted C_{1-4}-alkyl, optionally substituted C_{1-4}-acyloxy, nucleobases, DNA intercalators, photochemically active groups, thermochemically active groups, chelating groups, reporter groups, and ligands;

P designates the radical position for an internucleoside linkage to a succeeding monomer, or a 5'-terminal group, such internucleoside linkage or 5'-terminal group optionally including the substituent R^5 or equally applicable the substituent R^{5*};

P* designates an internucleoside linkage to a preceding monomer, or a 3'-terminal group;

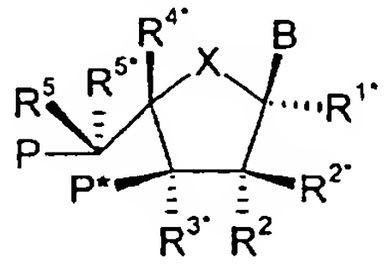
R^{2*} and R^{4*} designate biradicals consisting of 1-4 groups/atoms selected from -C(R^aR^b)-, -C(R^a)=C(R^a)-, -C(R^a)=N-, -O-, -Si(R^a)$_2$-, -S-, -SO_2-, -N(R^a)-, and >C=Z,

wherein Z is selected from -O-, -S-, and -N(R^a)-, and R^a and R^b each is independently selected from hydrogen, optionally substituted C_{1-12}-alkyl, optionally substituted C_{2-12}-alkenyl, optionally substituted C_{2-12}-alkynyl, hydroxy, C_{1-12}-alkoxy, C_{2-12}-alkenyloxy, carboxy, C_{1-12}-alkoxycarbonyl, C_{1-12}-alkylcarbonyl, formyl, aryl, aryloxy-carbonyl, aryloxy, arylcarbonyl, heteroaryl, heteroaryloxy-carbonyl, heteroaryloxy, heteroarylcarbonyl, amino, mono- and di(C_{1-6}-alkyl)amino, carbamoyl, mono- and di(C_{1-6}-alkyl)-amino-carbonyl, amino-C_{1-6}-alkyl-aminocarbonyl, mono- and di(C_{1-6}-alkyl)amino-C_{1-6}-alkyl-aminocarbonyl, C_{1-6}-alkyl-carbonylamino, carbamido, C_{1-6}-alkanoyloxy, sulphono, C_{1-6}-alkylsulphonyloxy, nitro, azido, sulphanyl, C_{1-6}-alkylthio, halogen, DNA intercalators, photochemically active groups, thermochemically active groups, chelating groups, reporter groups, and ligands, where aryl and heteroaryl may be optionally substituted, and where

4. An oligomer according to claim 2, wherein the oligonucleotide comprises at least 7, preferably at least 9, in particular at least 11, especially at least 13 successive L-ribo-LNA monomers.

5. An oligomer according to claim 2, wherein all nucleoside monomers of an oligomer are L-ribo-LNA.

6. An oligomer according to any of the claims 1-5, wherein the L-ribo-LNA(s) has/have the following formula Ia



Ia

wherein P, P*, B, X, R^{1*}, R^2, R^{2*}, R^{3*}, R^{4*}, R^5, and R^{5*} are as defined in claim 1.

7. An oligomer according to any of the claims 1-6, wherein X is selected from $-(CR^6R^{6*})-$, -O-, -S-, and $-N(R^{N*})-$, preferably -O-, -S-, and $-N(R^{N*})-$, in particular -O-.

8. An oligomer according to any of the claims 1-7, wherein the biradical constituted by R^{2*} and R^{4*} is selected from $-(CR^*R^*)_r-Y-(CR^*R^*)_s-$, $-(CR^*R^*)_r-Y-(CR^*R^*)_s-Y-$, $-Y-(CR^*R^*)_{r+s}-Y-$, $-Y-(CR^*R^*)_r-Y-(CR^*R^*)_s-$, $-(CR^*R^*)_{r+s}-$, -Y-, -Y-Y-, wherein each Y is independently selected from -O-, -S-, $-Si(R^*)_2-$, $-N(R^*)-$, >C=O, $-C(=O)-N(R^*)-$, and $-N(R^*)-C(=O)-$, each R^* is independently selected from hydrogen, halogen, azido, cyano, nitro, hydroxy, mercapto, amino, mono- or di(C_{1-6}-alkyl)amino, optionally substituted C_{1-6}-alkoxy, optionally substituted C_{1-6}-alkyl, DNA intercalators, photochemically active groups, thermochemically active groups, chelating groups, reporter groups, and ligands, and/or two adjacent (non-geminal) R^* may together designate a double bond, and each of r and s is 0-4 with the proviso that the sum r+s is 1-4.

9. An oligomer according to claim 8, wherein the biradical is selected from -Y-, $-(CR^*R^*)_{r+s}-$, $-(CR^*R^*)_r-Y-(CR^*R^*)_s-$, and $-Y-(CR^*R^*)_{r+s}-Y-$, wherein each of r and s is 0-3 with the proviso that the sum r+s is 1-4.

10. An oligomer according to claim 9, wherein the biradical is selected from -O-, -S-, -$N(R^*)$-, -$(CR^*R^*)_{r+s+1}$-, -$(CR^*R^*)_r$-O-$(CR^*R^*)_s$-, -$(CR^*R^*)_r$-S-$(CR^*R^*)_s$-, -$(CR^*R^*)_r$-$N(R^*)$-$(CR^*R^*)_s$-, -O-$(CR^*R^*)_{r+s}$-O-, -S-$(CR^*R^*)_{r+s}$-O-, -O-$(CR^*R^*)_{r+s}$-S-, -$N(R^*)$-$(CR^*R^*)_{r+s}$-O-, -O-$(CR^*R^*)_{r+s}$-$N(R^*)$-, -S-$(CR^*R^*)_{r+s}$-S-, -$N(R^*)$-$(CR^*R^*)_{r+s}$-$N(R^*)$-, -$N(R^*)$-$(CR^*R^*)_{r+s}$-S-, and -S-$(CR^*R^*)_{r+s}$-$N(R^*)$-, wherein each of r and s is 0-3 with the proviso that the sum r+s is 1-4, and where X is selected from -O-, -S-, and -$N(R^H)$- where R^H designates hydrogen or C_{1-4}-alkyl.

11. An oligomer according to claim 10, wherein X is O, R^2 is selected from hydrogen, hydroxy, and optionally substituted C_{1-6}-alkoxy, and R^{1*}, R^{3*}, R^6, and R^{5*} designate hydrogen.

12. An oligomer according to claim 11, wherein the biradical is selected from –O-, -$(CH_2)_{0-1}$-O-$(CH_2)_{1-3}$-, -$(CH_2)_{0-1}$-S-$(CH_2)_{1-3}$-, and -$(CH_2)_{0-1}$-$N(R^N)$-$(CH_2)_{1-3}$-, such as -O-CH_2-, -S-CH_2- and -$N(R^N)$-CH_2-.

13. An oligomer according to any of the claims 11-12, wherein B is selected from nucleobases.

14. An oligomer according to claim 8, wherein the biradical is -$(CH_2)_{2-4}$-.

15. An oligomer according to any of the claims 8-10, wherein one R^* is selected from hydrogen, hydroxy, optionally substituted C_{1-6}-alkoxy, optionally substituted C_{1-6}-alkyl, DNA intercalators, photochemically active groups, thermochemically active groups, chelating groups, reporter groups, and ligands, and any remaining substituents R^* are hydrogen.

16. An oligomer according to any of the claims 1-15, wherein any internucleoside linkage of the L-ribo-LNA(s) is selected from linkages consisting of 2 to 4, preferably 3, groups/atoms selected from -CH_2-, -O-, -S-, -NR^H-, >C=O, >C=NR^H, >C=S, -$Si(R'')_2$- -SO-, -$S(O)_2$-, -$P(O)_2$-, -P(O,S)-, -$P(S)_2$-, -PO(R'')-, -$PO(OCH_3)$-, and -$PO(NHR^H)$-, where R^H is selected form hydrogen and C_{1-4}-alkyl, and R'' is selected from C_{1-6}-alkyl and phenyl.

17. An oligomer according to claim 16, wherein any internucleoside linkage of the L-ribo-LNA(s) is selected from -CH_2-CH_2-CH_2-, -CH_2-CO-CH_2-, -CH_2-CHOH-CH_2-, -O-CH_2-O-,

-O-CH_2-CH_2-, -O-CH_2-CH=, -CH_2-CH_2-O-, -NR^H-CH_2-CH_2-, -CH_2-CH_2-NR^H-, -CH_2-NR^H-CH_2-, -O-CH_2-CH_2-NR^H-, -NR^H-CO-O-, -NR^H-CO-NR^H-, -NR^H-CS-NR^H-, -NR^H-C(=NR^H)-NR^H-, -NR^H-CO-CH_2-NR^H-, -O-CO-O-, -O-CO-CH_2-O-, -O-CH_2-CO-O-, -CH_2-CO-NR^H-, -O-CO-NR^H-, -NR^H-CO-CH_2-, -O-CH_2-CO-NR^H-, -O-CH_2-CH_2-NR^H-, -CH=N-O-, -CH_2-NR^H-O-, -CH_2-O-N=, -CH_2-O-NR^H-, -CO-NR^H-CH_2-, -CH_2-NR^H-O-, -CH_2-NR^H-CO-, -O-NR^H-CH_2-, -O-NR^H-, -O-CH_2-S-, -S-CH_2-O-, -CH_2-CH_2-S-, -O-CH_2-CH_2-S-, -S-CH_2-CH=, -S-CH_2-CH_2-, -S-CH_2-CH_2-O-, -S-CH_2-CH_2-S-, -CH_2-S-CH_2-, -CH_2-SO-CH_2-, -CH_2-SO_2-CH_2-, -O-SO-O-, -O-$S(O)_2$-O-, -O-$S(O)_2$-CH_2-, -O-$S(O)_2$-NR^H-, -NR^H-$S(O)_2$-CH_2-, -O-$S(O)_2$-CH_2-, -O-$P(O)_2$-O-, -O-P(O,S)-O-, -O-$P(S)_2$-O-, -S-$P(O)_2$-O-, -S-P(O,S)-O-, -S-$P(S)_2$-O-, -O-$P(O)_2$-S-, -O-P(O,S)-S-, -O-$P(S)_2$-S-, -S-$P(O)_2$-S-, -S-P(O,S)-S-, -S-$P(S)_2$-S-, -O-PO(R")-O-, -O-$PO(OCH_3)$-O-, -O-$PO(BH_3)$-O-, -O-$PO(NHR^N)$-O-, -O-$P(O)_2$-NR^H-, -NR^H-$P(O)_2$-O-, -O-P(O,NR^H)-O-, and -O-$Si(R")_2$-O-.

18. An oligomer according to any of the claims 1-17, wherein each of the substituents R^{1*}, R^2, R^{3*}, R^5, R^{5*}, R^6 and R^{6*} of the L-ribo-LNA(s), which are present, is independently selected from hydrogen, optionally substituted C_{1-6}-alkyl, optionally substituted C_{2-6}-alkenyl, hydroxy, C_{1-6}-alkoxy, C_{2-6}-alkenyloxy, carboxy, C_{1-6}-alkoxycarbonyl, C_{1-6}-alkylcarbonyl, formyl, amino, mono- and di(C_{1-6}-alkyl)amino, carbamoyl, mono- and di(C_{1-6}-alkyl)-amino-carbonyl, C_{1-6}-alkyl-carbonylamino, carbamido, azido, C_{1-6}-alkanoyloxy, sulphono, sulphanyl, C_{1-6}-alkylthio, DNA intercalators, photochemically active groups, thermochemically active groups, chelating groups, reporter groups, and ligands, and halogen, where two geminal substituents together may designate oxo, and where R^{N*}, when present and not involved in a biradical, is selected from hydrogen and C_{1-4}-alkyl.

19. An oligomer according to any of the claims 1-18, wherein X is selected from -O-, -S-, and -NR^{N*}-, and each of the substituents R^{1*}, R^2, R^{3*}, R^5, R^{5*}, R^6 and R^{6*} of the L-ribo-LNA(s), which are present, designate hydrogen.

20. An oligomer according to any of the claims 1-19, wherein P is a 5'-terminal group selected from hydrogen, hydroxy, optionally substituted C_{1-6}-alkyl, optionally substituted C_{1-6}-alkoxy, optionally substituted C_{1-6}-alkylcarbonyloxy, optionally substituted aryloxy, monophosphate, diphosphate, triphosphate, and -W-A', wherein W is selected from -O-, -S-, and -N(R^H)- where R^H is selected from hydrogen and C_{1-6}-alkyl, and where A' is selected from DNA intercalators, photochemically active groups, thermochemically active groups, chelating groups, reporter groups, and ligands.

21. An oligomer according to any of the claims 1-20, wherein P* is a 3'-terminal group selected from hydrogen, hydroxy, optionally substituted C_{1-6}-alkoxy, optionally substituted C_{1-6}-alkylcarbonyloxy, optionally substituted aryloxy, and -W-A', wherein W is selected from -O-, -S-, and -N(R^H)- where R^H is selected from hydrogen and C_{1-6}-alkyl, and where A' is selected from DNA intercalators, photochemically active groups, thermochemically active groups, chelating groups, reporter groups, and ligands.

22. An oligomer according to any of the claims 1-21, having the following formula III:

$$G\text{-}[Nu\text{-}L]_{n(0)}\text{-}\{[(L\text{-ribo-LNA})\text{-}L]_{m(q)}\text{-}[Nu\text{-}L]_{n(q)}\}_q\text{-}G^* \qquad III$$

wherein

q is 1-50;

each of n(0), .., n(q) is independently 0-10000;

each of m(1), .., m(q) is independently 1-10000;

with the proviso that the sum of n(0), .., n(q) and m(1), .., m(q) is 2-15000;

G designates a 5'-terminal group;

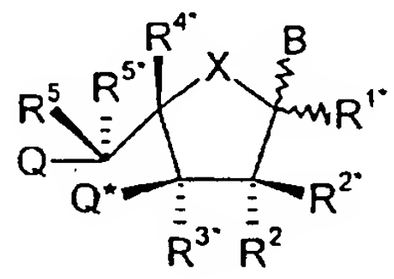
each Nu independently designates a nucleoside selected from naturally occurring nucleosides and nucleoside analogues;

each L-ribo-LNA independently designates a nucleoside analogue;

each L independently designates an internucleoside linkage between two groups selected from Nu and L-ribo-LNA, or L together with G* designates a 3'-terminal group; and

each L-ribo-LNA-L independently designates a nucleoside analogue of the general formula I.

23. A nucleoside analogue (L-ribo-LNA) of the general formula II



II

wherein the substituent B is selected from nucleobases, DNA intercalators, photochemically active groups, thermochemically active groups, chelating groups, reporter groups, and ligands;

X is selected from -O-, -S-, -N(R^{N*})-, and -C(R^6R^{6*})-;

each of Q and Q˙ is independently selected from hydrogen, azido, halogen, cyano, nitro, hydroxy, Prot-O-, Act-O-, mercapto, Prot-S-, Act-S-, C_{1-6}-alkylthio, amino, Prot-N(R^H)-, Act-N(R^H)-, mono- or di(C_{1-6}-alkyl)amino, optionally substituted C_{1-6}-alkoxy, optionally substituted C_{1-6}-alkyl, optionally substituted C_{2-6}-alkenyl, optionally substituted C_{2-6}-alkenyloxy, optionally substituted C_{2-6}-alkynyl, optionally substituted C_{2-6}-alkynyloxy, monophosphate, diphosphate, triphosphate, DNA intercalators, photochemically active groups, thermochemically active groups, chelating groups, reporter groups, ligands, carboxy, sulphono, hydroxymethyl, Prot-O-CH_2-, Act-O-CH_2-, aminomethyl, Prot-N(R^H)-CH_2-, Act-N(R^H)-CH_2-, carboxymethyl, sulphonomethyl, where Prot is a protection group for -OH, -SH, and -NH(R^H), respectively, Act is an activation group for -OH, -SH, and -NH(R^H), respectively, and R^H is selected from hydrogen and C_{1-6}-alkyl; and

R^{2*} and R^{4*} together designate a biradical selected from -O-, -(CR^*R^*)$_{r+s+1}$-, -(CR^*R^*)$_r$-O-(CR^*R^*)$_s$-, -(CR^*R^*)$_r$-S-(CR^*R^*)$_s$-, -(CR^*R^*)$_r$-N(R^*)-(CR^*R^*)$_s$-, -O-(CR^*R^*)$_{r+s}$-O-, -S-(CR^*R^*)$_{r+s}$-O-, -O-(CR^*R^*)$_{r+s}$-S-, -N(R^*)-(CR^*R^*)$_{r+s}$-O-, -O-(CR^*R^*)$_{r+s}$-N(R^*)-, -S-(CR^*R^*)$_{r+s}$-S-, -N(R^*)-(CR^*R^*)$_{r+s}$-N(R^*)-, -N(R^*)-(CR^*R^*)$_{r+s}$-S-, and -S-(CR^*R^*)$_{r+s}$-N(R^*)-;

wherein each R^* is independently selected from hydrogen, halogen, azido, cyano, nitro, hydroxy, mercapto, amino, mono- or di(C_{1-6}-alkyl)amino, optionally substituted C_{1-6}-alkoxy, optionally substituted C_{1-6}-alkyl, DNA intercalators, photochemically active groups, thermochemically active groups, chelating groups, reporter groups, and ligands, and/or two adjacent (non-geminal) R^* may together designate a double bond, and each of r and s is 0-3 with the proviso that the sum r+s is 1-4;

each of the present substituents R^{1*}, R^2, R^{3*}, R^5, R^{5*}, R^6, and R^{6*} is independently selected from hydrogen, optionally substituted C_{1-12}-alkyl, optionally substituted C_{2-12}-alkenyl, optionally substituted C_{2-12}-alkynyl, hydroxy, C_{1-12}-alkoxy, C_{2-12}-alkenyloxy, carboxy, C_{1-12}-alkoxycarbonyl, C_{1-12}-alkylcarbonyl, formyl, aryl, aryloxy-carbonyl, aryloxy, arylcarbonyl, heteroaryl, heteroaryloxy-carbonyl, heteroaryloxy, heteroarylcarbonyl, amino, mono- and di(C_{1-6}-alkyl)amino, carbamoyl, mono- and di(C_{1-6}-alkyl)-amino-carbonyl, amino-C_{1-6}-alkyl-aminocarbonyl, mono- and di(C_{1-6}-alkyl)amino-C_{1-6}-alkyl-aminocarbonyl, C_{1-6}-alkyl-carbonylamino, carbamido, C_{1-6}-alkanoyloxy, sulphono, C_{1-6}-alkylsulphonyloxy, nitro,

azido, sulphanyl, C_{1-6}-alkylthio, halogen, DNA intercalators, photochemically active groups, thermochemically active groups, chelating groups, reporter groups, and ligands, where aryl and heteroaryl may be optionally substituted, and where two geminal substituents together may designate oxo, thioxo, imino, or optionally substituted methylene, or together may form a spiro biradical consisting of a 1-5 carbon atom(s) alkylene chain which is optionally interrupted and/or terminated by one or more heteroatoms/groups selected from -O-, -S-, and -(NR^N)- where R^N is selected from hydrogen and C_{1-4}-alkyl, and where two adjacent (non-geminal) substituents may designate an additional bond resulting in a double bond; and R^{N*}, when present and not involved in a biradical, is selected from hydrogen and C_{1-4}-alkyl;

and basic salts and acid addition salts thereof;

and with the proviso that any chemical group (including any nucleobase), which is reactive under the conditions prevailing in oligonucleotide synthesis, is optionally functional group protected.

24. A nucleoside analogue according to claim 23, wherein the group B is selected from nucleobases and functional group protected nucleobases.

25. A nucleoside analogue according to any of the claims 23-24, wherein X is selected from -O-, -S-, and -N(R^{N*})-.

26. A nucleoside analogue according to any of the claims 23-25, wherein each of the substituents R^{1*}, R^2, R^{3*}, R^5, R^{5*}, R^6, and R^{6*}, which are present, is independently selected from hydrogen, optionally substituted C_{1-6}-alkyl, optionally substituted C_{2-6}-alkenyl, hydroxy, C_{1-6}-alkoxy, C_{2-6}-alkenyloxy, carboxy, C_{1-6}-alkoxycarbonyl, C_{1-6}-alkylcarbonyl, formyl, amino, mono- and di(C_{1-6}-alkyl)amino, carbamoyl, mono- and di(C_{1-6}-alkyl)-amino-carbonyl, C_{1-6}-alkyl-carbonylamino, carbamido, azido, C_{1-6}-alkanoyloxy, sulphono; sulphanyl, C_{1-6}-alkylthio, DNA intercalators, photochemically active groups, thermochemically active groups, chelating groups, reporter groups, ligands, and halogen, where two geminal substituents together may designate oxo, and where R^{N*}, when present and not involved in a biradical, is selected from hydrogen and C_{1-4}-alkyl, with the proviso that any hydroxy, amino, mono(C_{1-6}-alkyl)amino, sulfanyl, and carboxy is optionally protected.

27. A nucleoside analogue according to any of the claims 23-26, each of the substituents R^{1*}, R^2, R^{3*}, R^5, R^{5*}, R^6 and R^{6*}, which are present, designate hydrogen.

28. A nucleoside analogue according to any of the claims 23-27, wherein Q is independently selected from hydrogen, azido, halogen, cyano, nitro, hydroxy, Prot-O-, mercapto, Prot-S-, C_{1-6}-alkylthio, amino, Prot-N(R^H)-, mono- or di(C_{1-6}-alkyl)amino, optionally substituted C_{1-6}-alkoxy, optionally substituted C_{1-6}-alkyl, optionally substituted C_{2-6}-alkenyl, optionally substituted C_{2-6}-alkenyloxy, optionally substituted C_{2-6}-alkynyl, optionally substituted C_{2-6}-alkynyloxy, monophosphate, diphosphate, triphosphate, DNA intercalators, photochemically active groups, thermochemically active groups, chelating groups, reporter groups, ligands, carboxy, sulphono, hydroxymethyl, Prot-O-CH_2-, aminomethyl, Prot-N(R^H)-CH_2-, carboxymethyl, sulphonomethyl, where Prot is a protection group for -OH, -SH, and -NH(R^H), respectively, and R^H is selected from hydrogen and C_{1-6}-alkyl; and

Q^* is selected from hydrogen, azido, halogen, cyano, nitro, hydroxy, Act-O-, mercapto, Act-S-, C_{1-6}-alkylthio, amino, Act-N(R^H)-, mono- or di(C_{1-6}-alkyl)amino, optionally substituted C_{1-6}-alkoxy, optionally substituted C_{1-6}-alkyl, optionally substituted C_{2-6}-alkenyl, optionally substituted C_{2-6}-alkenyloxy, optionally substituted C_{2-6}-alkynyl, optionally substituted C_{2-6}-alkynyloxy, DNA intercalators, photochemically active groups, thermochemically active groups, chelating groups, reporter groups, ligands, carboxy, sulphono, where Act is an activation group for -OH, -SH, and -NH(R^H), respectively, and R^H is selected from hydrogen and C_{1-6}-alkyl.

29. A nucleoside analogue according to any of the claims 23-28, wherein X is O, R^2 selected from hydrogen, hydroxy, and optionally substituted C_{1-6}-alkoxy, and R^{1*}, R^3, R^5, and R^{5*} designate hydrogen.

30. A nucleoside analogue according to claims 23-29, wherein the biradical is selected from -O-, -$(CH_2)_{0-1}$-O-$(CH_2)_{1-3}$-, -$(CH_2)_{0-1}$-S-$(CH_2)_{1-3}$-, and -$(CH_2)_{0-1}$-N(R^N)-$(CH_2)_{1-3}$-.

31. A nucleoside analogue according to claim 30, wherein the biradical is selected from -O-CH_2-, -S-CH_2- and -N(R^N)-CH_2-.

32. A nucleoside analogue according to claim 23-31, wherein the biradical is -$(CH_2)_{2-4}$-, preferably –$(CH_2)_2$-.

33. A conjugate of a L-ribo-LNA modified oligonucleotide (an oligomer) as defined in any of the claims 1-26 and a compound selected from proteins, amplicons, enzymes, polysaccharides, antibodies, haptens, peptides, and PNA.

34. The use of an L-ribo-LNA as defined in any of the claims 23-32 for the preparation of an L-ribo-LNA modified oligonucleotide (an oligomer) according to any of the claims 1-34.

35. The use according to claim 33, wherein the incorporation of L-ribo-LNA modulates the ability of the oligonucleotide to act as a substrate for nucleic acid active enzymes.

36. The use of a L-ribo-LNA as defined in any of the claims 23-32 for the preparation of a conjugate of an L-ribo-LNA modified oligonucleotide and a compound selected from proteins, amplicons, enzymes, polysaccharides, antibodies, haptens, peptides, and PNA.

37. The use of a L-ribo-LNA as defined in any of the claims 23-32 as a substrate for enzymes active on nucleic acids.

38. The use according to claim 37, wherein the L-ribo-LNA is used as a substrate for DNA and RNA polymerases.

39. The use of an L-ribo-LNA as defined in any of the claims 23-32 as a therapeutic agent.

49. The use of an L-ribo-LNA as defined in any of the claims 23-32 for diagnostic purposes.

40. The use of one or more L-ribo-LNA as defined in any of the claims 23-32 in the construction of solid surface onto which LNA modified oligonucleotides of different sequences are attached .

41. The use of L-ribo-LNA modified oligomers (ribozymes) as defined in any of the claims 1-22 in the sequence specific cleavage of target nucleic acids.

42. The use of a L-ribo-LNA modified oligonucleotide (an oligomer) as defined in any of the claims 1-22 in therapy, such as an antisense, an antigene or a gene activating therapeutic.

43. The use according to claim 42, wherein the LNA modified oligonucleotide recruits RNAseH.

44. The use of complexes of more than one L-ribo-LNA modified oligonucleotide (an oligomer) said oligonucleotides being defined according to any of the claims 1-22, in therapy, such as as an antisense, an antigene or gene activating therapeutic.

45. The use of an α-L-ribo-LNA modified oligonucleotide (an oligomer) as defined in any of the claims 6-22 in therapy, wherein the α-L-ribo-LNA modified oligonucleotide specifically interact with RNA selected from the group consisting of tRNAs, rRNAs, snRNAs and scRNAs thereby inhibiting any of the following cellular processes selected from the group consisting of translation, RNA splicing, RNA processing, and other essential cellular processes.

46. The use of an L-ribo-LNA modified oligonucleotide (an oligomer) as defined in any of the claims 6-22 in diagnostics suc as for the isolation, purification, amplification, detection, identification, quantification, or capture of natural or synthetic nucleic acids.

47. The use of an α-L-ribo-LNA modified oligonucleotide (an oligomer) as defined in any of the claims 6-22 in diagnostics such as for the isolation, purification, amplification, detection, identification, quantification, or capture of natural or synthetic nucleic acids, said α-L-ribo-LNA modified oligonucleotide being able to discriminate between RNA and DNA thereby selectively hybridizing to the target RNA.

48. The use according to any of claims 46 or 47, wherein the oligonucleotide comprises a photochemically active group, a thermochemically active group, a chelating group, a reporter group, or a ligand that facilitates the direct or indirect detection of the oligonucleotide or the immobilisation of the oligonucleotide onto a solid support.

49. The use according to claim 48, wherein the photochemically active group, the thermochemically active group, the chelating group, the reporter group, or the ligand includes a spacer (K), said spacer comprising a chemically cleavable group.

50. The use according to claim 49, wherein the photochemically active group, the thermochemically active group, the chelating group, the reporter group, or the ligand is attached via the biradical (i.e. as $R^{\bullet}$) of at least one of the LNA(s) of the oligonucleotide.

51. The use according to claim 50 for capture and detection of naturally occurring or synthetic double stranded or single stranded nucleic acids such as RNA or DNA.

52. The use according to claim 47 for purification of naturally occurring double stranded or single stranded nucleic acids such as RNA or DNA.

53. The use of an L-ribo-LNA modified oligonucleotide (an oligomer) as defined in any of the claims 1-22 as an aptamer in molecular diagnostics.

54. The use of an L-ribo-LNA modified oligonucleotide (an oligomer) as defined in any of the claims 1-22 as an aptamer in RNA mediated catalytic processes.

55. The use of an L-ribo-LNA modified oligonucleotide (an oligomer) as defined in any of the claims 1-22 as an aptamer in specific binding of antibiotics, drugs, amino acids, peptides, structural proteins, protein receptors, protein enzymes, saccharides, polysaccharides, biological cofactors, nucleic acids, or triphosphates.

56. The use of an L-ribo-LNA modified oligonucleotide (an oligomer) as defined in any of the claims 1-22 as an aptamer in the separation of enantiomers from racemic mixtures by stereospecific binding.

57. The use of a L-ribo-LNA modified oligonucleotide (an oligomer) as defined in any of the claims 1-22 for the labelling of cells.

58. The use of an L-ribo-LNA modified oligonucleotide (an oligomer) as defined in any of the claims 1-22 to hybridise to non-protein coding cellular RNAs, such as tRNA, rRNA, snRNA and scRNA, *in vivo* or *in-vitro*.

59. The use of a L-ribo-LNA modified oligonucleotide (an oligomer) as defined in any of the claims 1-22 in the construction of an oligonucleotide comprising a fluorophor and a quencher, positioned in such a way that the hybridised state of the oligonucleotide can be distinguished from the unbound state of the oligonucleotide by an increase in the fluorescent signal from the probe.

60. A kit for the isolation, purification, amplification, detection, identification, quantification, or capture of natural or synthetic nucleic acids, the kit comprising a reaction body and one or more L-ribo-LNA modified oligonucleotides (oligomer) as defined in any of the claims 1-22.

61. A kit for the isolation, purification, amplification, detection, identification, quantification, or capture of natural or synthetic nucleic acids, the kit comprising a reaction body and one or more L-ribo-LNAs as defined in any of the claims 23-32.

62. A kit according to claim 60 or 61, wherein the L-ribo-LNAs are immobilised onto said reactions body.

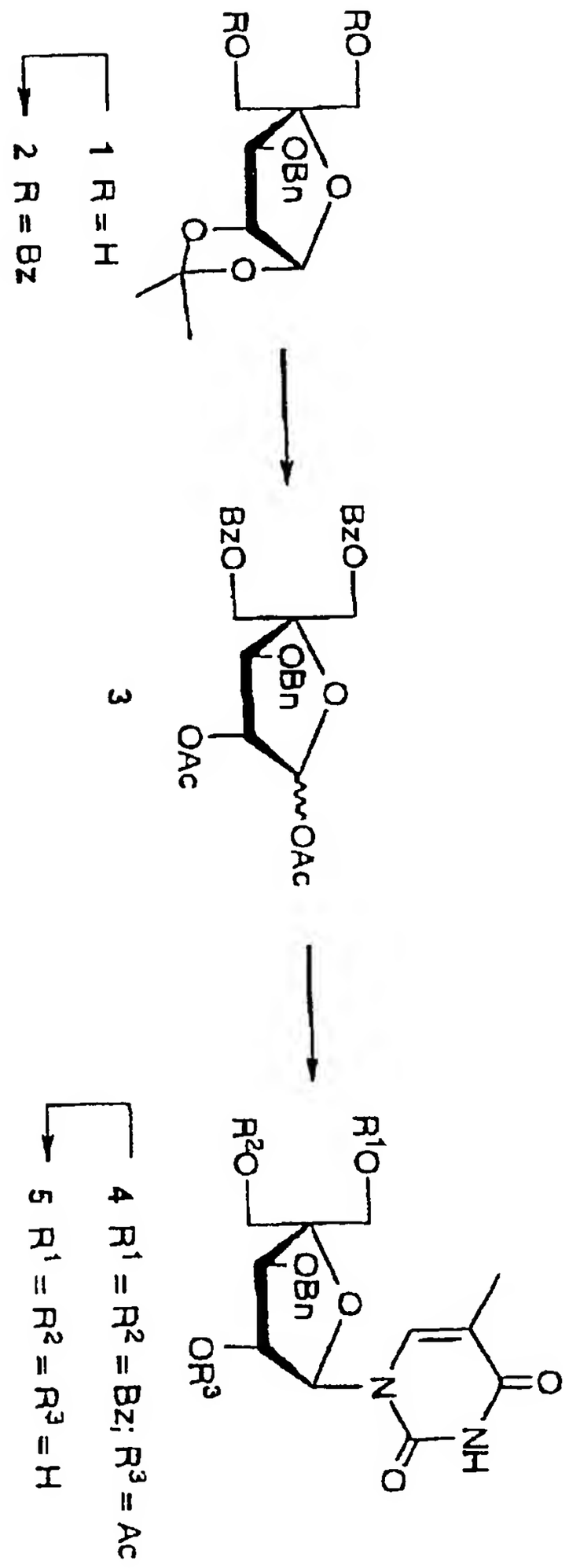

RO
RO
OBn
O
O
O
1 R=H
2 R=Bz
BzO
BzO
OBn
O
OAc
OAc
3
R1O
R2O
OBn
O
OR3
N
O
NH
O
4 R1 = R2 = Bz; R3 = Ac
5 R1 = R2 = R3 = H

Fig. 1

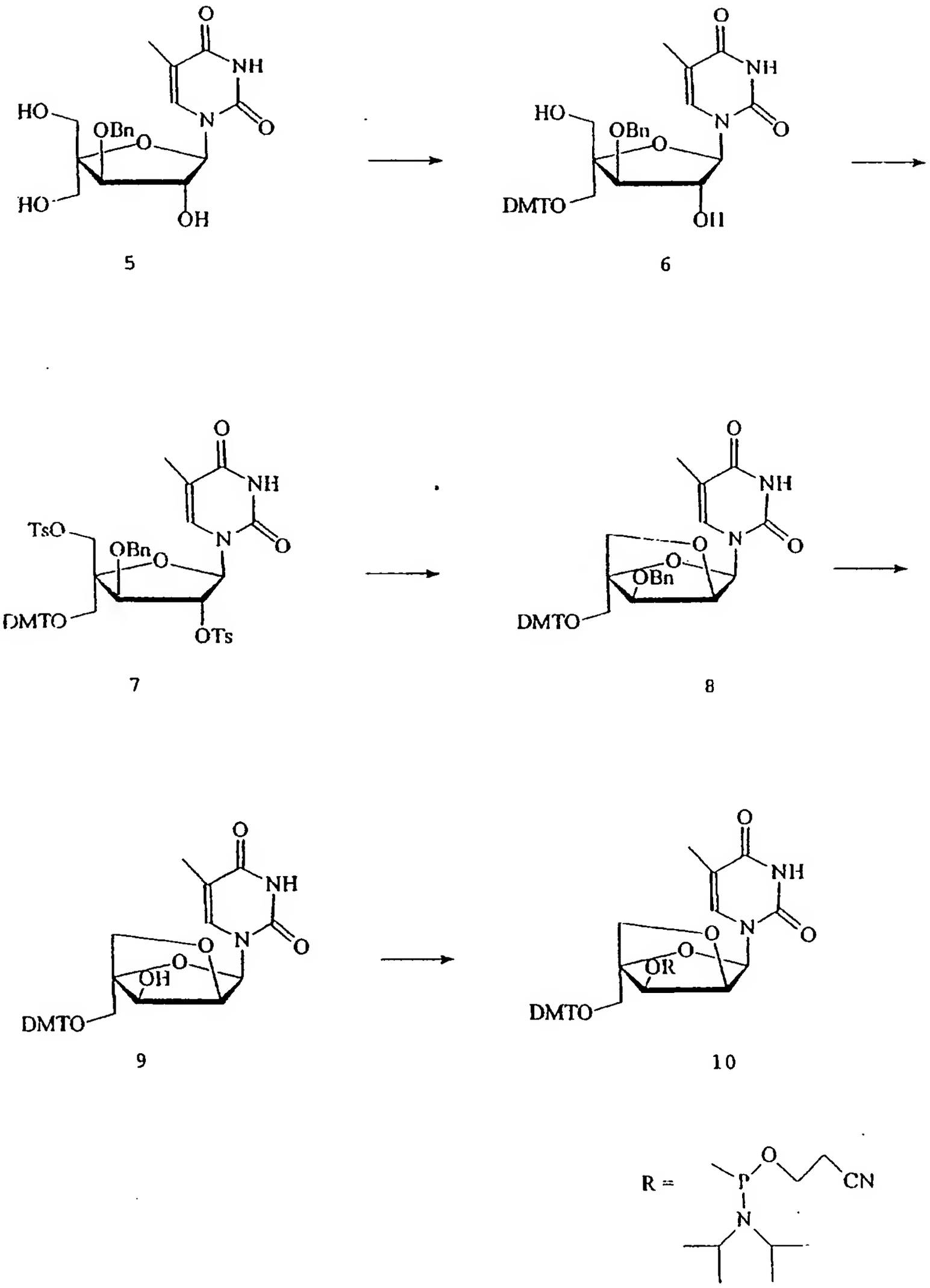


Fig. 2

Fig. 3

Fig. 3

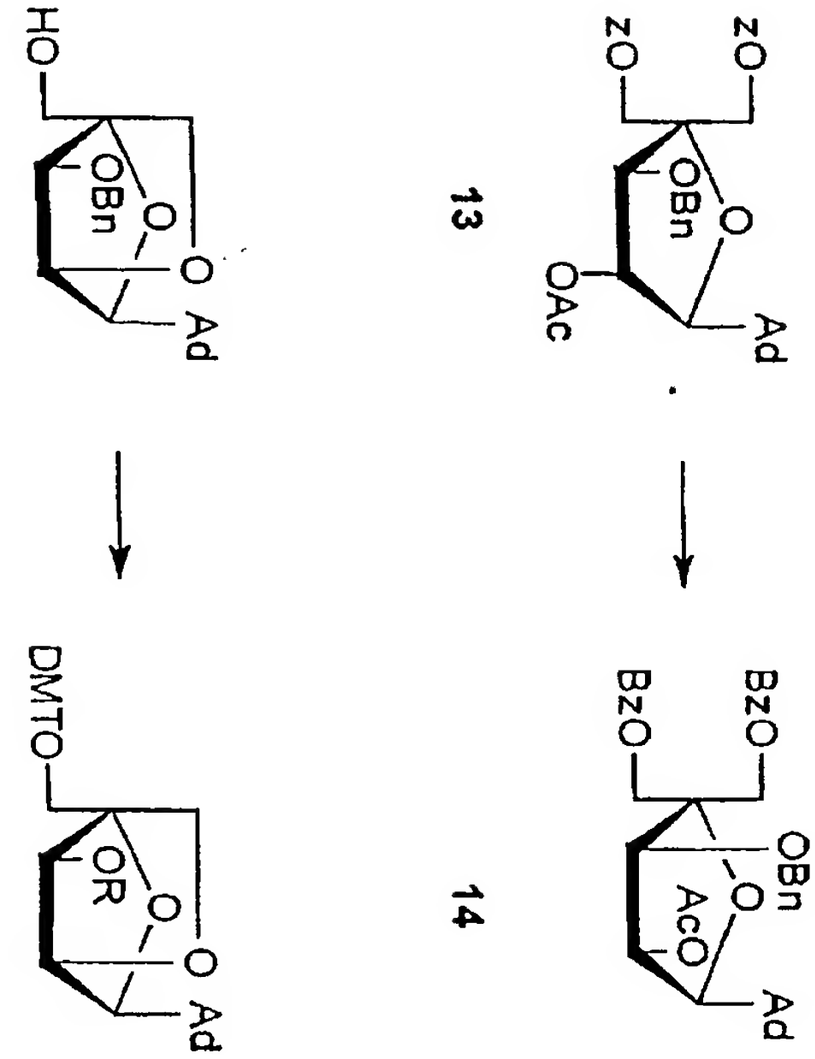

zO
zO
OBn
O
OAc
Ad
13
BzO
BzO
OBn
O
AcO
Ad
14
HO
OBn
O
O
Ad
15
DMTO
OR
O
O
Ad
16
Ad = 6-N-benzoyladenin-9-yl
R = NC(CH2)2OP(NiPr2)

Fig. 4